Diet as a Factor in Length of Life and in Structure and Composition of Tissues of the Rat with Aging

Home Economics Research Report No. 24

Agricultural Research Service

UNITED STATES DEPARTMENT OF AGRICULTURE

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by

Mildred Adams

Human Nutrition Research Division

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Preface

This research was done as part of a project supported by an allotment made by the Secretary of Agriculture from Special Research Funds (Bankhead-Jones Act of June 29, 1935).

Staff members in the Human Nutrition Research Division responsible for the results reported in this publication were as follows:

Mildred Adams, Research Chemist. Organization and evaluation of data; preparation of report.

Elizabeth C. Hartman,[1] Nutrition Physiologist. Initiation of this investigation; general supervision for the longevity studies.

Anna M. Allen Durand, Medical Officer (Histology). Evaluation of histological findings in the tissues.

Murray Fisher, Biologist. General supervision of experimental animals; collection of data on organ weights; evaluation of gross findings at necropsy; preparation of tissues for histological examination.

Doris D. Taylor, Nutrition Specialist, and Emily S. Conway, Biological Aid (Gen.). Chemical analysis of diets, livers, kidneys, and serum cholesterol. Mrs. Conway also assisted in compiling and tabulating the data.

Hazel E. Hildebrand, Nutrition Specialist. Calorie determinations.

Florence L. Lakshmanan, Biochemist, and Donald Higginbotham,[2] Biochemist. Electrophoretic analysis of serum.

Mention of specific products throughout this publication does not imply recommendation by the U.S. Department of Agriculture over other products of a similar nature not mentioned.

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Diet as a Factor in Length of Life and in Structure and Composition of Tissues of the Rat with Aging

By Mildred Adams

Human Nutrition Research Division, Agricultural Research Service

Early investigations with the laboratory rat as experimental animal have dealt with nutritional factors important for normal growth and development of the young animal, and have provided much information of basic importance to human nutrition. In recent years, increasing emphasis has been placed on the need for information concerning the requirements of adult animals at various stages of their lifespan.

In this laboratory, investigations have been underway for several years to determine the influence of various dietary combinations on the length of life and on the appearance of changes in the structure and composition of tissues of the rat. A preliminary report (*39*) [3] from this laboratory has indicated that the substitution of cooked dried egg for 25 percent of a nutritionally adequate basal diet accelerated development of degenerative changes in tissues of the adult rat. When the diet consisted of 100 percent whole egg, the tissue changes observed were less severe and occurred later in life, suggesting that an imbalance of nutrients rather than egg itself may have been responsible for the adverse results with the diet containing 25 percent egg.

In this publication are reported results of extensive investigations using the rat as the experimental animal and dealing with the influence of diet on survival and some of the factors, including diet, that may affect the presence or absence of pathological lesions and the size and proximate composition of selected organs. Included also are results showing the influence of age and diet on cholesterol and on various protein fractions in the blood serum.

The majority of the experimental diets were modifications of a relatively simple diet composed chiefly of semipurified components. In one group of diets, protein and fat were varied by replacing 20 to 25 percent of the semipurified diet with egg, milk, beef, or peanut butter. In a second group of diets, the source and level of protein remained constant but the kind and level of fat varied. The fats were hydrogenated vegetable oil (HVO), lard, and butter; the levels were 8 and 16 percent. In addition, limited data are reported on the effect of supplementing the diet containing 25 percent egg with various B vitamins alone or in combination. The results of feeding diets containing relatively high levels of egg yolk or egg white or consisting solely of whole egg or egg yolk are also included.

[3] Italic numbers in parentheses refer to Literature Cited, p. 93.

Experimental

Description and Management of Animals

A strain of rats (BHE) developed in this laboratory by crossing Albino (Yale strain obtained from Columbia University) and black and white hooded rats (Pennsylvania State College) served as the chief source of the experimental animals. The litters included white, black, or black and white rats. The parent stock animals were raised on a standard pelleted ration[4] that is employed in our breeding laboratories and has been found to be efficient for growth, reproduction, and lactation.

[4] Animal Foundation Laboratory Diet, Standard Brands, Inc., N.Y.

A small group of young from a colony of Wistar animals [5] raised on a different stock ration [6] was also investigated to determine if the dietary response would differ with another strain of rat. To obtain information on the possible carryover effect of the diet of the parents, a few BHE rats were raised on the stock diet on which the parents of the Wistar rats had been maintained and the response of their young to diet was determined. Male rats were used throughout these investigations because preliminary studies had indicated that males were more susceptible than females to certain dietary regimens that caused early death and accelerated degenerative changes in the tissues.

Rats were placed on the experimental diets at weaning, when they were between 21 and 24 days of age. The majority of weanling rats weighed between 40 and 50 grams. Except for one series, the animals were maintained on a constant diet throughout life. To determine whether the response to diet could be influenced by the age at which consumption of the diet was begun, the influence of changing the dietary regimen at 250 days of age was investigated. For each series, only those litters were used that contained enough males to permit placing one littermate on each experimental diet in the series. The average initial weights of the animals fed each diet were kept as nearly uniform as possible.

All animals were kept in an air-conditioned laboratory maintained at a temperature of 78°–82° F. and at a relative humidity which averaged 37 percent, although not rigorously controlled. The animals were fed ad libitum and had access to water at all times. The animals fed the pelleted stock ration were taken directly from the stock colony. They were housed five to six to a cage, and no data were obtained on their food intake. Rats fed the experimental diets were housed in individual metal cages with raised screen floors. When urine collections were made, the animals were transferred to wire metabolism cages with half-inch mesh bottoms supported above glass funnels 9 inches in diameter.

The rats fed experimental diets were weighed daily for the first 2 to 3 weeks and weekly thereafter. For many of the series, food intake records were maintained. Food intake was recorded daily during the first 2 to 3 weeks and twice weekly throughout the remainder of their life. Scattered food was collected and weighed at the time of recording food intake. Observations were made regularly of the general physical condition of the animals.

[5] Kindly supplied to us by Arthur M. Hartman, Animal Husbandry Research Division, Agricultural Research Service, Beltsville, Md.

[6] Stock colony ration consisted of: yellow cornmeal, 68.5 percent; linseed oil meal, 14.0 percent; meat scrap, 9.0 percent; commercial casein, 4.0 percent; alfalfa meal, 2.0 percent; bone meal, 2.0 percent; and sodium chloride, 0.5 percent. Supplements of lettuce and carrots were fed once a week.

For some experiments, urine was collected over a 7-hour period, during which time the animals had access to water but not to food; for others, urine was collected over a 16- to 17-hour period, with the rats having access to both food and water. To collect the urine samples, 50-ml. glass centrifuge tubes containing toluene were placed under glass funnels. Plugs of extremely fine wire mesh were placed in the neck of each funnel to screen out feces, feed, and hair.

To determine the changes that occur with aging, some animals were sacrificed at scheduled ages while they were maintaining or gaining weight and were showing no obvious signs of illness. Other animals designated for survival studies were continued on the experimental diets until they became obviously ill. These rats reduced their food intake or stopped eating entirely, they became listless, and their coats became rough. These animals consistently lost weight and occasionally suffered from obvious respiratory disturbances.

It was not possible, without 24-hour vigilance, to keep animals until they died naturally and still obtain tissues suitable for microscopic examination. Thus the results of the longevity studies were dependent upon arbitrary decisions as to when the rats were approaching death. At first, animals were allowed to continue until extremely ill, with weight losses often equal to as much as one-third of their maximum body weight. Unexpected deaths resulted in failure to obtain tissues that were suitable for histologic or chemical analysis because of post-mortem changes. Later, to avoid loss of suitable tissues, rats for longevity studies were sacrificed as soon as there was consistent weight loss for at least 3 weeks and/or when weight loss exceeded 50 grams.

During the early investigations, rats were sacrificed by injecting 0.5 ml. of 2 percent solution of sodium amytal per 100 grams body weight without a preliminary fasting period. When the criteria under investigation were extended to include measurements of various serum protein fractions, it became necessary to sacrifice the animals after a 17-hour fast and to obtain blood samples by cardiac puncture.

Diets

The semipurified diet, which served as a basis for most of the experimental diets, consisted of:

Ingredient	*Percent*
Casein [1]	16
Lactalbumin [2]	8
Yeast [3]	10
Salt mixture [4]	4
Hydrogenated vegetable oil (HVO) [5]	8
Sucrose	52
Celluflour	2

[1] High-nitrogen, acid-washed, edible product, from Sheffield Farms Co.
[2] Labco Lactalbumin containing some lactose, from Borden Co.
[3] Dried brewer's yeast, type 200B, from Standard Brands, Inc.
[4] Source: Osborne and Mendel (*149*).
[5] Crisco, from Procter & Gamble Co., Cincinnati, Ohio.

The following supplements per animal were fed with all diets: Percomorph oil, 2 drops weekly, supplying 395 I.U. of vitamin A and 56 I.U. of vitamin D daily; *dl*-alpha-tocopherol acetate, 36 mg. in 0.01 ml. of cottonseed oil weekly, or 5.1 mg. daily; fresh kale, 10 grams twice weekly.

In table 1 are summarized the modifications of this diet and the codes used in the text. Egg, milk, beef, or peanut butter were substituted for part of the semipurified diet in such a way that all of these diets contained approximately the same amount of protein and of fat. The fat content of these diets was approximately twice that present in the semipurified diet. The foods themselves provided the extra fat in SPE or SPPB diets. Extra fat beyond that in the food to be studied was provided by butterfat in the SPM diet and by suet in the SPB diet. The diets containing 8 and 16 percent HVO, lard, or butter were all identical except for the kind and level of fat and the reduced level of sucrose when 16 percent fat was used. Other modifications of the SP 8 HVO diet and the diets consisting of 100 percent whole egg, 100 percent egg yolk, or 97 percent egg yolk with added salt mixture (3 percent) were included in an attempt to elucidate the possible role of egg fat or protein, or both in the response of rats to the SPE diet. In addition, the effect of adding certain supplements (selected vitamins and cholesterol) to the SPE diet was investigated to obtain some information on the possibility that an imbalance of nutrients was a factor in the response of rats to this diet.

To each 100 grams of SPE diet the following supplements were added:

- Choline, 0.5 gram
- Vitamin B_{12}, 0.01 mg.
- Choline, 0.5 gram + vitamin B_{12}, 0.01 mg.
- Vitamin B_6, 0.5 mg.
- Choline, 0.5 gram + vitamin B_6, 0.5 mg.
- Choline, 0.5 gram + vitamin B_6, 0.5 mg. + vitamin B_{12}, 0.01 mg.
- Cholesterol, 0.46 gram
- Cholesterol, 1.38 grams
- Ascorbic acid, 0.2 gram
- Cholesterol, 0.46 gram + ascorbic acid, 0.2 gram.

The dry ingredients for the experimental diets were weighed in the proportions already described and placed in a Hobart mixer, with the sucrose added last. The melted fat was poured onto the sucrose, and the diet was prepared by blending in the mixer. This procedure resulted in a more uniform product than that obtained when the fat was added to the mixed dry ingredients.

Dried whole egg, before being added to the SPE diet, was blended with water, then cooked in a double boiler and stirred constantly until the egg was firm and dry in appearance; the lumps were broken in a Foley mill, dried at 150° F. with forced draught, and ground.

For the SPB diet, the beef was cut in 4-inch cubes and cooked in water, in a large steam-jacketed kettle, for 30 minutes. The beef was then ground, dried, and reground to produce a fine powder for blending with other ingredients of the diet. The beef suet was rendered and refrigerated until used.

TABLE 1.—*Diet codes and description of experimental diets*

Code	Description
SP 8 HVO	Semipurified.
SPE	SP 8 HVO, 75 percent + whole egg, commercially dried,[1] 25 percent.
SPM	SP 8 HVO, 75 percent + skim milk,[2] 15 percent + butterfat,[3] 10 percent.
SPB	SP 8 HVO, 75 percent + beef,[4] 15 percent + beef suet,[5] 10 percent.
SPPB	SP 8 HVO, 80 percent + peanut butter,[6] 20 percent.
SP 16 HVO	SP 8 HVO with HVO level increased to 16 percent and sucrose decreased to 44 percent.
SP 8 lard	SP 8 HVO with lard [7] replacing HVO.
SP 16 lard	SP 16 HVO with lard replacing HVO.
SP 8 butter	SP 8 HVO with butter [3] replacing HVO.
SP 16 butter	SP 16 HVO with butter replacing HVO.
SPa 16 HVO	SP 16 HVO with casein increased to 20 percent, lactalbumin increased to 10 percent, and sucrose decreased to 38 percent.
SPb 8 HVO	SP 8 HVO with casein increased to 20 percent, lactalbumin increased to 10 percent, and sucrose decreased to 46 percent.
SPEW	SP 8 HVO, 86 percent, except HVO, 15 percent; sucrose, 41 percent; and egg white,[8] 10 percent.
SPEY	SP 8 HVO, 66 percent, except no HVO; sucrose, 43 percent; and egg yolk,[8] 30 percent.
SPW 8 HVO	SP 8 HVO with egg white, 24 percent, replacing casein and lactalbumin.
E100	Whole commercially dried egg, 100 percent.
Y100	Fresh egg yolk, cooked and dried, 100 percent.
Y97+salt mixture	Y100, 97 percent, + salt mixture, 3 percent.

[1] Whole egg, spray dried, from Henningsen Bros., Inc.
[2] Starlac, from Borden Co.
[3] Butter washed free of protein and salt. Animal Husbandry Research Division, Agricultural Research Service, Beltsville, Md.
[4] Beef rounds from dual-purpose cattle with all visible fat removed. Meat Quality Laboratory, Animal Husbandry Research Division, Agricultural Research Service, Beltsville, Md.
[5] Beef suet from kidney area. Meat Quality Laboratory, Animal Husbandry Research Division, Agricultural Research Service, Beltsville, Md.
[6] Peanut butter from two sources: (1) Prepared from roasted white Spanish peanuts by Southern Utilization Research Laboratory in accordance with directions published by the Laboratory (*18*). The peanut butter contained 1.2 to 1.3 percent commercial hydrogenated peanut oil (Onesta Hardener, Procter and Gamble Co.). (2) Peter Pan Peanut Butter containing salt was used for most of the investigations.
[7] Lard. Animal Husbandry Research Division, Agricultural Research Service, Beltsville, Md.
[8] Prepared in the laboratory from hard-boiled eggs.

For diets containing egg yolks or whites, hard-boiled eggs were used. Yolks and whites were separated, ground, and dried at 150° F. The whites were reground after drying. The diet containing a high level of egg yolk was reground after mixing.

Cholesterol or vitamin supplements, except for choline, were added dry and mixed with the SPE diet by blending in the Hobart mixer. Choline was dissolved in water first and then added to the dry ingredients before final blending.

The diets were prepared fresh at least every 2 weeks and were kept refrigerated until used. All of the fats and the ingredients containing fat were kept under refrigeration. Aliquots of the dietary ingredients and of the experimental diets were kept for chemical analysis.

The dietary ingredients and the experimental diets were analyzed for moisture, nitrogen, fat, and ash. Moisture was determined by drying in a vacuum oven at 70° C. Ash values were obtained by incineration in a muffle furnace at 575° C. Nitrogen was determined by the Kjeldahl-Wilfarth-Gunning method using mercury as a catalyst *(12)* and distilling into boric acid *(175)*. Protein values were obtained by applying to the nitrogen values the factors indicated. Fat was determined by a modification [7] of the AOAC acid hydrolysis procedure *(13)*. Carbohydrate values were calculated by subtracting the weight of fat, protein, and ash from the total dry weight. Mineral components were determined by spectrochemical analysis for the various dietary ingredients and for most of the diets. Those diets not actually analyzed were calculated from the values for the dietary ingredients. Gross calorie values were obtained for some of the diets by use of the Parr Bomb Adiabatic Calorimeter; others were calculated by application of appropriate gross calorie values for the proteins, fats, and carbohydrates which they contained. The values for thiamine, riboflavin, niacin, pyridoxine, folic acid, pantothenic acid, vitamin B_{12}, choline, essential and nonessential amino acids, fatty acids, and cholesterol were all calculated.

Criteria for Evaluating Response to Diet

Physical measurements, histological examination of the tissues, and limited chemical analyses of liver, kidney, blood serum, and urine provided the criteria used for evaluating the changes occurring in the rat with age and/or with diet. Measurements obtained on BHE rats fed the stock diet provided information on the characteristics of the rats that served as a source of the experimental animals. A group of rats fed SP 8 HVO diet was included for comparative purposes in each of the experimental series alinged chiefly with modifications of this diet. Rats fed SPE diet served as a basis of comparison for the series of rats fed SPE diets containing various supplements and for those fed high levels of egg or egg fractions.

Weight and intake data provided the information needed to compare the rate of growth, maximum weight attained, and efficiency of food utilization. The influence of age and/or diet on the size of the organs was determined by weighing immediately, at the time of sacrifice, the liver, right kidney, right adrenal, and right lobe of the thyroid.

Also, at the time of sacrifice, any gross abnormalities in the animals were noted. The left kidney and adrenal, the left lobe of the thyroid, and part of the median lobe of the liver (approximately 20 percent by weight of the total liver) were fixed in 10 percent formalin to preserve these tissues for histological examination. Other tissues prepared routinely for histological examination included the heart, lungs, aorta, salivary glands, spleen, pancreas, and testes. These tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

The methods used for rating the microscopic findings and the results of gross and microscopic examination of the tissues are being reported in detail elsewhere *(54)*. This report includes only those phases of the histological findings that are related to the chemical investigations reported, and deals chiefly with the kidney and liver. Damage to the liver and kidney was rated arbitrarily 1 to 4, with 4 the most extensive damage.

Chemical analyses included determinations of moisture, fat, protein, and ash in livers and kidneys, cholesterol and protein fractions in blood serum, and coagulable protein in urine. Most of the chemical analyses were obtained for rats that were fasted before sacrifice. Immediately after removal of the section of liver retained for histological examination, the weight of the remaining portion was recorded and both the liver and kidney were stored frozen, for chemical analysis. Homogenates of these organs were prepared by use of a Virtis homogenizer, and the various analyses were carried out on weighed aliquots of these homogenates. The values reported for the liver content are for the whole liver, based on the assumption that the composition of the sections analyzed was similar to that of sections removed for histological examination.

During the early series, small kidneys were pooled in order to obtain sufficient material for

[7] Unpublished.

duplicate analysis. Once the conditions had been established for producing a uniform homogenate from the kidney, the results from chemical analyses showed excellent reproducibility. It then seemed preferable to analyze individual kidneys to permit a direct comparison of the results with the other measurements under consideration and to avoid masking differences that may result from average values. Only one analytical value could be obtained for kidneys weighing 2 grams or less. Duplicate values were obtained when sufficient material was available.

The methods for analysis of tissues were the same as those used for the food samples, except that fat determinations were done by the AOAC acid hydrolysis method (*13*). The coagulable protein in the freshly collected urine samples was determined by precipitating with 5 percent acetic acid (*63*) and weighing the washed, dried precipitate. Blood serum samples collected at the time of sacrifice were stored, refrigerated, at 4° C. until analyzed for cholesterol and for the various protein components. Serum cholesterol was determined by the direct method of Zlatkis, Zak, and Boyle (*192*). It has been established (*108*) that the values obtained by the use of the direct method are high, but further investigations in this laboratory have indicated that the relationships reported here are substantially correct. Serum protein components were determined by electrophoretic analysis, using the method of Tiselius (*183*) as modified by Longsworth and Jacobsen (*118*).

Results and Discussion

Composition of Experimental Diets

On the basis of current information, the majority of the diets investigated provided adequate amounts of the nutrients essential for the growing rat and amounts generally considered adequate or more than adequate for maintenance of the adult animal. Admittedly, our knowledge of the requirements of the rat at various stages of life is far from complete.

The analyzed or calculated values for the nutrient content of the diets are summarized in tables 2 through 7. Corresponding information for the ingredients used in preparing these diets is summarized in tables 75 through 78 of the appendix. To facilitate evaluation of the adequacy of the experimental diets under consideration, the amounts of nutrients suggested as requirements for the growing rat and for the adult rat have been included when such information was available. Many factors may influence the requirement for a specific nutrient such as the heredity of rats under investigation and the proportion of other dietary components. Most of the values for nutrient requirement that are recorded represent the average of results obtained by several investigators, and have been limited to requirements of rats fed relatively simple diets similar to the semipurified diet used in the investigations reported in this bulletin.

Protein

The protein content (table 2) of all but three of the experimental diets was within the range of 25 to 30 percent of the diet—an amount suggested for optimum growth of the rat. Two diets, SPM with 23.8 percent protein and SPW with 24.6 percent protein, were only slightly below the 25-percent level; the third diet, consisting of 100 percent whole egg, provided a considerable excess. The stock diet contained most of the essential amino acids in relatively small amounts when compared with the other diets under investigation but still provided sufficient amounts to meet requirements. The diet consisting of 100 percent egg with its high protein content supplied the essential amino acids in amounts considerably in excess of requirements. The other diets provided from two to four times the amount of the essential amino acids required for the growing rat, and still greater excesses for the adult animal. Considering tryptophan as the base line, no marked differences in the amino acid patterns of these diets were observed except for stock and SPW diets. The stock diet contained relatively small amounts of tryptophan when compared with the other amino acids. Methionine and cystine were high in SPW diet in relation to its tryptophan content.

Fat

Data for the fatty acid composition of the diets are presented in table 3 as a percentage of the dietary fat and in table 4 as a percentage of the diet. No data were available for the fatty acid composition of yeast fat, and the results recorded are exclusive of the fat from this source. The small amount of fat in the yeast (0.5 percent or less of the diet) would not be sufficient to change materially the major characteristics of the dietary fat. Table 3 also includes the iodine values for the dietary fats, and table 4 includes the cholesterol content of the diets.

The iodine values of the food fats (table 3) ranged from 33.4 for SP 8 butter or SP 16 butter to 87.8 for SPPB diet, reflecting the relatively high linoleic acid content of peanut butter. Of the fat in SP 8 HVO, SP 16 HVO, SPa 16 HVO, SPb 8 HVO, SPEW, and SPW diets, more than 60 percent was oleic acid. Saturated fatty acids of chain length 16 or less accounted for more than 40 percent of the fat in the diets containing 8 and 16 percent butter.

The concentration of fat in these diets (table 4) differed widely, with the smallest amount 6.3 percent in the stock diet and the largest amount 58 percent in the diet consisting of 100 percent egg yolk. Although the daily intake of the latter diet was less, fat consumption was higher on this diet than on any of the other experimental

TABLE 2. *Protein and amino acid[1] content per 100 grams of diet, and suggested requirements[2][3] for the rat*

Diet	Nitrogen[4]	Protein factor[1]	Protein	Tryptophan	Threonine	Isoleucine	Leucine	Lysine	Methionine	Cystine	Phenylalanine	Tyrosine	Valine	Arginine	Histidine	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine
	Grams		*Grams*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Stock	4.71	6.25	29.4	0.22	0.93	1.66	1.64	1.08	0.29	0.13	1.10	(5)	1.30	1.43	0.40	(5)	(5)	(5)	(5)	(5)	(5)
SP 8 HVO	3.99	6.33	25.3	.41	1.20	1.58	2.55	2.07	.67	.33	1.24	1.27	1.70	1.04	.68	1.26	2.25	4.95	0.55	2.12	1.49
SPE	4.82	6.30	30.3	.50	1.48	1.96	2.94	2.30	.87	.52	1.61	1.45	2.14	1.55	.79	.95	2.51	5.16	.83	2.09	1.10
SPM	3.75	6.34	23.8	.38	1.14	1.52	2.44	1.96	.63	.30	1.19	1.22	1.64	.98	.65	1.13	2.08	4.96	.52	2.18	1.43
SPB	4.76	6.30	30.0	.44	1.40	1.78	2.85	2.55	.78	.40	1.40	1.34	1.91	1.52	.91	1.61	2.76	5.44	1.12	2.16	1.60
SPPB	4.13	6.13	25.3	.39	1.12	1.51	2.41	1.87	.59	.36	1.30	1.23	1.66	1.48	.69	1.22	2.65	5.13	.78	2.01	1.59
SP 16 HVO	3.99	6.33	25.3	.41	1.20	1.58	2.55	2.07	.67	.33	1.24	1.27	1.70	1.04	.68	1.26	2.25	4.95	.55	2.12	1.49
SP 8 lard	3.99	6.33	25.3	.41	1.20	1.58	2.55	2.07	.67	.33	1.24	1.27	1.70	1.04	.68	1.26	2.25	4.95	.55	2.12	1.49
SP 16 lard	3.99	6.33	25.3	.41	1.20	1.58	2.55	2.07	.67	.33	1.24	1.27	1.70	1.04	.68	1.26	2.25	4.95	.55	2.12	1.49
SP 8 butter	3.99	6.33	25.3	.41	1.20	1.58	2.55	2.07	.67	.33	1.24	1.27	1.70	1.04	.68	1.26	2.25	4.95	.55	2.12	1.49
SP 16 butter	3.99	6.33	25.3	.41	1.20	1.58	2.55	2.07	.67	.33	1.24	1.27	1.70	1.04	.68	1.26	2.25	4.95	.55	2.12	1.49
SPa 16 HVO	4.68	6.33	29.6	.49	1.43	1.91	3.10	2.49	.81	.40	1.50	1.53	2.05	1.24	.82	1.48	2.67	6.01	.62	2.60	1.78
SPb 8 HVO	4.68	6.33	29.6	.49	1.43	1.91	3.10	2.49	.81	.40	1.50	1.53	2.05	1.24	.82	1.48	2.67	6.01	.62	2.60	1.78
SPEW	4.78	6.31	30.1	.47	1.38	1.87	2.89	2.25	.88	.48	1.58	1.42	2.08	1.37	.76	1.69	2.56	5.37	.77	2.12	1.97
SPEY	4.25	6.30	26.8	.40	1.24	1.59	2.44	1.96	.67	.37	1.22	1.25	1.74	1.31	.66	.84	1.99	4.35	.68	1.81	1.78
SPW 8 HVO	3.94	6.25	24.6	.38	1.12	1.52	2.07	1.53	1.70	.53	1.45	1.12	1.82	1.39	.56	1.85	2.11	3.44	1.00	.96	1.82
E100	7.49	6.25	46.8	.77	2.33	3.11	4.12	3.00	1.47	1.09	2.70	2.01	3.48	3.07	1.12	(5)	3.28	5.79	1.66	1.98	3.93
Y100	4.73	6.25	29.5	.43	1.50	1.81	2.49	1.95	.76	.50	1.30	1.37	2.03	2.05	.67	(5)	1.63	3.54	1.04	1.31	2.62
Range for diets:																					
High	7.49	------	46.8	.77	2.33	3.11	4.12	3.00	1.70	1.09	2.70	2.01	3.48	3.07	1.12	1.85	3.28	6.01	1.66	2.60	3.93
Low	3.75	------	23.8	.22	.93	1.51	1.64	1.08	.29	.13	1.10	1.12	1.30	.98	.40	.84	1.63	3.44	.52	.96	1.10
Estimated requirements:																					
Young	------	------	25-30	.2	.5	.5	.8	1.0	.4	------	.7	------	.7	.2	.4	------	------	------	------	------	------
Adult	------	------	------	.1	.28	.54	.28	.20	.30	------	.22	------	.37	0	.11	------	------	------	------	------	------

[1] Except for stock diet, values calculated from individual foods. (See appendix table 75.) Stock diet values supplied by manufacturer.

[2] Source: McCoy (*122*). [3] Source: Almquist, (*8*, pp. 136-167). [4] Analyzed values. [5] No data reported.

TABLE 3.—*Fatty acid content per 100 grams of diet* [1] *and iodine value of dietary fat*

Diet	Saturated fatty acids										Unsaturated fatty acids						Iodine value
	Total	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	Total	Mono-		Di-	Tri-	All	
												C_{10}–C_{16}	C_{18}	C_{18}	C_{18}	C_{20}–C_{22}	
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*Grams*	*Grams*	
SP 8 HVO	25. 9	0. 3	0. 2	0. 1	0. 1	0. 4	1. 3	15. 2	6. 9	1. 5	69. 7	0. 5	62. 2	6. 8	0. 2	(2)	70. 5
SPE	28. 7	. 1	. 1	(2)	(2)	. 1	. 7	20. 7	6. 3	. 6	66. 8	3. 6	53. 3	7. 4	1. 0	1. 5	78. 8
SPM	44. 2	2. 0	1. 1	. 5	. 9	2. 3	5. 3	21. 7	10. 0	. 6	51. 4	1. 4	44. 8	4. 4	. 6	. 2	48. 1
SPB	39. 5	. 1	. 1	(2)	(2)	. 1	. 5	23. 5	14. 7	. 5	54. 4	. 2	50. 4	3. 7	. 1	(2)	55. 3
SPPB	25. 8	. 1	. 1	(2)	. 1	. 2	1. 2	12. 9	6. 2	5. 1	69. 8	. 2	47. 0	22. 5	. 1	. 1	87. 8
SP 16 HVO	24. 3	. 2	. 1	(2)	. 1	. 2	1. 0	14. 7	6. 6	1. 5	71. 3	. 4	63. 7	7. 0	. 2	(2)	72. 4
SP 8 lard	40. 0	. 3	. 2	. 1	. 1	. 4	. 8	30. 3	7. 9	------	55. 6	. 2	44. 6	9. 8	. 6	. 4	61. 0
SP 16 lard	39. 2	. 2	. 1	(2)	. 1	. 2	. 4	30. 5	7. 7	------	56. 4	. 1	45. 2	10. 1	. 6	. 4	62. 4
SP 8 butter	56. 2	3. 0	1. 7	. 8	1. 3	3. 6	7. 9	25. 8	12. 0	------	39. 4	2. 0	33. 4	2. 9	. 8	. 3	33. 4
SP 16 butter	56. 2	3. 0	1. 7	. 8	1. 3	3. 6	7. 9	25. 8	12. 0	------	39. 4	2. 0	33. 4	2. 9	. 8	. 3	33. 4
SPa 16 HVO	24. 7	. 2	. 1	(2)	. 1	. 2	1. 0	14. 8	6. 7	1. 5	70. 9	. 4	63. 4	6. 9	. 2	(2)	71. 4
SPb 8 HVO	26. 6	. 4	. 2	. 1	. 2	. 4	1. 5	15. 5	7. 0	1. 4	69. 0	. 5	61. 6	6. 7	. 3	(2)	69. 7
SPEW	24. 2	. 1	. 1	(2)	. 1	. 2	. 9	14. 6	6. 6	1. 6	71. 6	. 4	63. 9	7. 0	. 2	. 1	72. 6
SPEY	31. 3	. 1	. 1	(2)	(2)	. 1	. 6	24. 2	6. 1	------	64. 2	5. 4	47. 3	7. 7	1. 4	2. 4	82. 4
SPW 8 HVO	22. 6	------	------	------	------	------	. 6	14. 0	6. 3	1. 6	73. 0	. 3	65. 4	7. 2	. 2	------	74. 6
E100	30. 5	------	------	------	------	------	. 4	24. 2	5. 9	------	65. 1	5. 5	47. 8	7. 8	1. 4	2. 5	84. 0
Y100	30. 5	------	------	------	------	------	. 4	24. 2	5. 9	------	65. 1	5. 5	47. 8	7. 8	1. 4	2. 5	84. 0
Range for diets:																	
High	56. 2	3. 0	1. 7	. 8	1. 3	3. 6	7. 9	30. 5	14. 7	5. 1	73. 0	5. 5	65. 4	22. 5	1. 4	2. 5	87. 8
Low	22. 6	. 1	. 1	(2)	(2)	. 1	. 4	12. 9	5. 9	. 5	39. 4	. 1	33. 4	2. 9	. 1	(2)	33. 4

[1] Values for diets calculated from composition of fat in individual foods. (See appendix table 76.) No data available for yeast; fatty acids and iodine values are exclusive of yeast fat.
[2] Less than 0.05 gram.

TABLE 4.—*Fat, fatty acid, and cholesterol content per 100 grams of diet*

Diet	Total fat [1]	Saturated fatty acids [2]										Unsaturated fatty acids [2]						Cholesterol [3]
		Total	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	Total	Mono-		Di-	Tri-	All C_{20}–C_{22}	
													C_{16}–C_{18}	C_{18}	C_{18}	C_{18}		
	Grams	*Grams*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*Grams*	*Mg.*
Stock	6.3																	
SP 8 HVO	9.4	2.30	0.03	0.02	(4)	(4)	0.04	0.12	1.35	0.61	0.13	6.19	0.04	5.52	0.60	0.02	(4)	10
SPE	17.4	4.98	.02	.02	(4)	(4)	.02	.12	3.59	1.09	.10	11.58	.62	9.24	1.28	.17	0.26	478
SPM	17.2	7.45	.34	.18	0.08	0.15	.39	.89	3.64	1.68	.10	8.66	.24	7.55	.74	.10	.03	36
SPB	19.3	7.46	.02	.02	(4)	(4)	.02	.09	4.44	2.78	.09	10.27	.04	9.52	.70	.02	(4)	42
SPPB	17.9	4.43	.02	.02	(4)	.02	.03	.21	2.21	1.06	.88	11.98	.03	8.07	3.86	.02	.02	8
SP 16 HVO	17.1	4.10	.03	.02	(4)	.02	.03	.17	2.48	1.11	.25	12.04	.07	10.75	1.18	.03	(4)	10
SP 8 lard	9.4	3.55	.03	.02	(4)	(4)	.04	.07	2.69	.70		4.84	.02	3.96	.87	.05	.04	20
SP 16 lard	17.1	6.62	.03	.02	(4)	.02	.03	.07	5.15	1.30		9.52	.02	7.63	1.70	.10	.07	29
SP 8 butter	9.4	4.99	.27	.15	.07	.12	.32	.70	2.29	1.07		3.50	.20	2.97	.26	.07	.03	29
SP 16 butter	17.1	9.49	.52	.29	.14	.22	.61	1.33	4.36	2.03		6.65	.34	5.64	.49	.14	.05	48
SPa 16 HVO	17.1	4.22	.03	.02	(4)	.02	.03	.17	2.53	1.14	.26	12.12	.07	10.84	1.18	.03	(4)	13
SPb 8 HVO	9.4	2.42	.04	.02	(4)	.02	.04	.14	1.41	.64	.13	6.27	.05	5.60	.61	.03	(4)	13
SPEW	16.3	3.81	.02	.02	(4)	.02	.03	.14	2.30	1.04	.25	11.28	.06	10.06	1.10	.03	.02	9
SPEY	18.4	5.63	.02	.02	(4)	(4)	.02	.11	4.35	1.10		11.54	.97	8.50	1.38	.25	.43	841
SPW 8 HVO	8.6	1.81						.05	1.12	.50	.13	5.84	.02	5.23	.58	.02		0
E100	42.7	13.02						.17	10.33	2.52		27.80	2.35	20.41	3.33	.60	1.07	1,880
Y100	58.0	17.69						.23	14.04	3.42		37.76	3.19	27.72	4.52	.81	1.45	2,780
Range for diets:																		
High	58.0	17.69	.52	.29	.14	.22	.61	1.33	14.04	3.42	.88	37.76	3.19	27.72	4.52	.81	1.45	2,780
Low	6.3	1.81	.02	.02	(4)	(4)	.02	.05	1.12	.50	.09	3.50	.02	2.97	.26	.02	(4)	0

[1] Analyzed values, including yeast fat.
[2] Exclusive of fatty acids from yeast—values calculated from dietary ingredients. (See appendix table 76.)
[3] Calculated from ingredients. (See appendix table 76.)
[4] Less than 0.01 gram.

diets. The total fat content of the diet was, of course, a determining factor in the total content of the individual fatty acids and was responsible for the large amounts of oleic and linoleic acid in the diets composed entirely of whole egg or egg yolk. Although the linoleic acid content of the egg-yolk diet was higher than that of the SPPB diet, the total consumption of this fatty acid when egg yolk was fed was about 60 percent of the consumption for SPPB diet. The latter diet supplied about eight times as much linoleic acid as did the diet containing a comparable level of fat, chiefly as butter fat. Linoleic acid, the only fatty acid so far shown to be essential for the rat, was present in all of the diets in amounts sufficient to supply the 25 mg. per day reported (*122*) necessary to prevent the development of skin lesions in the young rat and to permit growth to proceed at a normal rate. Linoleic acid as well as other fatty acids may play a role in other phases of lipid metabolism, but the importance of specific fatty acid patterns in the nutrition of the rat has not yet been established.

The cholesterol content of the diets was relatively low, amounting to less than 50 mg. per 100 grams of diet except for those diets containing egg. The concentration of cholesterol was 478 mg. per 100 grams of SPE diet and was exceedingly high—2,780 mg. per 100 grams—in the diet consisting of 100 percent egg yolk.

Vitamins

The data in table 5 summarizing the vitamin content of the diets and the requirements for the growing rat indicate that all of the vitamins known to be required by the rat were supplied in ample amounts. The high level of some of the B vitamins in the semipurified diet and its modifications was provided by the brewer's yeast, which was present in all of these diets. Although the thiamine content of the stock diet and of the 100 percent whole-egg or egg-yolk diet was low in comparison with the other diets, it was sufficient to supply the amount of this vitamin considered essential.

Choline requirements depend on many factors (*35*) such as sex, strain, age, and dietary methionine, cystine, betaine, or cholesterol. Although 10 to 20 mg. daily have been fed by many investigators to supply the needs of the actively growing rat, this amount is considerably more than is necessary under some circumstances. In diets containing from 24 to 30 percent casein, chloine requirements are small (0 to 6 mg. daily). The requirement of the rat over 30 days of age is also small. According to Slanetz (*173*) only from 1 to 3 mg. daily are required by older rats. Although the level of choline was low in most of the experimental diets reported in this publication, the amount supplied should be adequate for the adult rat and even for the growing rat in view of the methionine content of these diets. Levels considerably in excess of requirements were provided by the diets containing egg or egg yolk unless the high cholesterol content of these diets increased the need for choline.

Vitamins A, D, and E were not incorporated into the diets, and the values recorded for these vitamins are in terms of daily intake. The chief source of vitamins A and D for all except rats fed the stock diet was the supplement of percomorph oil. Carotene from the kale supplement also contributed to the vitamin A potency of the diets. Egg and egg yolk were the only other foods to provide significant amounts of this vitamin. The supplement of *dl*-alpha-tocopherol acetate supplied generous amounts of vitamin E compared to reported requirements.

Nerurkar and Sahasrabudhe (*137*) reported that pure vitamin A is toxic to young male rats when given orally at a dose of 40,000 I.U. daily. When feeding was continued for 10 days, there was a gradual decrease in the percentage retention of calcium, phosphorus, and nitrogen. The toxic dose of vitamin D is generally high, but no exact data can be given. In man and dogs (*161, p. 430*), 20,000 I.U. daily may produce toxic symptoms. The amounts of vitamins A and D in the experimental diets under consideration in this publication were in excess of requirements, but they were considerably below the amounts that have been reported to be toxic for relatively short-term studies.

Minerals

The salt mixture used for preparing the semipurified diet was the chief source of minerals and, as seen in table 6, relatively small differences were observed in the ash content of these diets. The stock diet contained from two and a half to three times the ash content of the experimental diets, and large amounts of calcium and phosphorus in satisfactory proportions. Diets consisting of 100 percent egg or egg yolk were low in calcium, with a ratio of calcium to phosphorus below the desirable range. Potassium intakes were considerably in excess of requirements. Manganese tended to be low. Aluminum values have not been included. The were high and variable because of comtamination with aluminum from the grinders used in preparing these diets.

Calories

The data for the calorie values of the experimental diets are summarized in table 7. In the majority of the diets, sucrose was the chief carbohydrate. The cereal starches supplied most of the carbohydrate in the stock diet. Fat supplied less than 20 percent of the gross calories from the stock diet and from the various modifications of the semipurified diet containing approximately 9 percent of fat. The remaining diets, except for E100 and Y100, supplied 30 to 35 percent of the calories as fat. The calories from fat in diet Y100 were 74 percent of the total gross calories from this diet.

TABLE 5.—*Vitamin content of diets,*[1] *and suggested requirements for the rat*

Diet	Content per 100 grams of diet									Daily intake		
	Thiamine	Ribo-flavin	Niacin	Folic acid	Pyri-doxine	Panto-thenic acid	Vitamin B_{12}	Choline	Biotin	Vitamin A	Vitamin D	Vitamin E [2]
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*µg.*	*Mg.*	*µg.*	*I.U.*	*I.U.*	*Mg.*
Stock	0.23	1.06	3.26	0.17	0.28	0.77	2.42	131	15.0	200	>22	----
SP 8 HVO	6.01	.85	5.02	.21	.22	1.32	1.06	26	8.6	610	56	5.1
SPE	4.60	.90	3.82	.16	.30	2.82	3.30	486	17.7	750	56	5.1
SPM	4.56	.94	3.92	.16	.22	1.49	1.36	40	11.7	660	56	5.1
SPB	4.54	.71	5.60	.16	.39	1.25	.90	62	8.4	610	56	5.1
SPPB	4.84	.71	7.26	.18	.25	1.49	.85	46	14.9	610	56	5.1
SP 16 HVO	6.01	.85	5.02	.21	.22	1.32	1.06	26	8.6	610	56	5.1
SP 8 lard	6.01	.85	5.02	.21	.22	1.32	1.06	26	8.6	610	56	5.1
SP 16 lard	6.01	.85	5.02	.21	.22	1.32	1.06	26	8.6	610	56	5.1
SP 8 butter	6.01	.85	5.02	.21	.22	1.32	1.06	26	8.6	650	56	5.1
SP 16 butter	6.01	.85	5.02	.21	.22	1.32	1.06	26	8.6	690	56	5.1
SPa 16 HVO	6.01	.89	5.02	.21	.22	1.33	1.32	26	8.7	610	56	5.1
SPb 8 HVO	6.01	.89	5.02	.21	.22	1.33	1.32	26	8.7	610	56	5.1
SPEW	5.14	.94	4.36	.18	.19	1.19	.90	22	12.2	610	56	5.1
SPEY	4.14	.77	3.37	.15	.50	3.21	5.11	839	18.9	870	56	5.1
SPW 8 HVO	6.00	1.19	5.17	.21	.22	1.52	----	26	19.8	610	56	5.1
E100	.34	1.06	.20	.01	.54	7.34	10.0	1,864	45.0	980	56	5.1
Y100	.50	.66	.10	.03	1.18	7.92	14.7	2,740	44.0	1,050	56	5.1
Range for diets:												
High	6.01	1.19	7.26	.21	1.18	7.92	14.7	2,740	45.0	1,050	56	5.1
Low	.23	.66	.10	.01	.19	.77	.85	22	8.4	200	>22	5.1
Estimated requirements:												
Young rat	[3] .100, [4] .125	[4] .25, [3] .40	[4] 0	----	[3 4] .100	[4] 1.0	[5] 1.0	[6] 20–30	[7] 0, [8] 10–30	[4] 4	[9] 30	[4] .3 [2] 1.0

[1] Except for stock diet, values were calculated from ingredients. See appendix table 77. Data for stock diet supplied by manufacturer.

[2] Fed as supplement. In addition, approximately 1.5 mg. per day contributed by HVO in diets containing 8 percent of this fat, or 3 mg. when level was 16 percent. Diets containing peanut butter contributed approximately 1 mg. per day. Source: Deuel (*48*), calculated from data in table 1, page 690.

[3] Source: McCoy (*122*).

[4] Based on consumption of 10 grams daily. Source: Brown and Sturtevant (*35*).

[5] Personal communication with Arthur M. Hartman, Animal Husbandry Research Division, Agricultural Research Service, Beltsville, Md., from data indicating growth close to maximum with 1 µg. per 100 grams of diet based on 4-week growth rate of vitamin B_{12}-depleted rats.

[6] Sufficient to prevent renal lesions and fatty livers. See footnote 2. Requirements dependent on many factors, including sex, strain, and age. See also footnote 4.

[7] Not essential when casein is in the diet. See footnote 4.

[8] Source: Rosenburg (*161*, p. 478).

[9] Not required if calcium-phosphorus ratio in the diet is between 1:1 and 2:1, and 0.5 percent phosphorus is present. Outside this range, diet is improved by addition of 3 I.U. per gram of food, or 30 I.U. on basis of 10-gram intake. Source: McCoy (*122*, p. 99).

TABLE 6.—*Ash and mineral content per 100 grams of diet, and suggested requirements for the rat*

Diet	Ash [1]	Calcium	Phosphorus	Iron	Copper	Sodium	Potassium	Magnesium	Manganese	Boron	Ratio—Calcium: Phosphorus
	Grams	*Grams*	*Grams*	*Mg.*	*Mg.*	*Grams*	*Grams*	*Mg.*	*Mg.*	*Mg.*	
Stock [2]	10. 0	2. 17	1. 22	17	0. 7	0. 64	0. 59	290	3. 20	------	1. 8
SP 8 HVO [3]	3. 6	. 56	. 52	14	1. 5	. 16	. 78	83	. 66	0. 14	1. 1
SPE [3]	3. 1	. 41	. 51	14	. 9	. 22	. 68	72	. 51	. 13	. 8
SPM [3]	3. 9	. 58	. 42	12	. 8	. 18	. 81	57	. 46	. 12	1. 4
SPB [3]	3. 1	. 36	. 45	12	1. 1	. 13	. 77	67	. 45	. 17	. 8
SPPB [3]	3. 1	. 46	. 48	14	1. 0	. 15	. 95	110	1. 50	. 32	1. 0
SP 16 HVO [4]	3. 6	. 56	. 52	14	1. 5	. 16	. 78	83	. 66	. 14	1. 1
SP 8 lard [4]	3. 6	. 56	. 52	14	1. 5	. 16	. 78	83	. 66	. 14	1. 1
SP 16 lard [4]	3. 6	. 56	. 52	14	1. 5	. 16	. 78	83	. 66	. 14	1. 1
SP 8 butter [4]	3. 6	. 56	. 52	14	1. 5	. 16	. 78	83	. 66	. 14	1. 1
SP 16 butter [4]	3. 6	. 56	. 52	14	1. 5	. 16	. 78	83	. 66	. 14	1. 1
SPa 16 HVO [4]	4. 1	. 54	. 51	13	. 9	. 16	. 98	85	. 46	. 06	1. 1
SPb 8 HVO [4]	4. 1	. 54	. 51	13	. 9	. 16	. 98	85	. 46	. 06	1. 1
SPEW [4]	3. 3	. 47	. 43	11	. 7	. 22	. 93	82	. 40	. 06	1. 1
SPEY [4]	3. 3	. 43	. 54	11	. 6	. 14	. 69	61	. 34	. 06	. 8
SPW 8 HVO [4]	4. 4	. 54	. 44	12	. 7	. 35	1. 18	104	. 45	. 03	1. 2
E100 [3]	3. 5	. 21	. 62	8	. 4	. 40	. 50	42	. 18	. 17	. 3
Y100 [3]	3. 2	. 24	. 72	8	. 2	. 11	. 13	16	. 13	. 10	. 3
Range for diets:											
High	10. 0	2. 17	1. 22	17	1. 5	. 64	1. 18	290	3. 20	. 32	1. 8
Low	3. 1	. 21	. 42	8	. 2	. 11	. 13	16	. 13	. 03	. 3
Estimated requirements: [5]											
Young rat	------	. 50–. 60	. 45–. 55	2. 5	. 5–1. 0	. 07	. 15	5	[6] 5	. 008	1:1–2:1

[1] Analyzed values.
[2] Except for ash values, data supplied by manufacturer.
[3] Analyzed spectrochemically by A. W. Specht and J. W. Resnicky, Soil and Water Conservation Research Division, Agricultural Research Service, Beltsville, Md.
[4] Calculated from ingredients. See appendix table 78.
[5] Source: McCoy (*122*).
[6] Minimal requirements for reproduction.

TABLE 7.—*Calorie values per 100 grams of diet, and percentage of calories as carbohydrate, fat, and protein*

Diet	Heat of combustion	Percentage of gross calories as—		
		Carbohydrates	Fat	Protein
	Calories			
Stock	[1] 399	47	14	39
SP 8 HVO	[1] 470	50	19	31
SPE	[1] 520	35	32	33
SPM	[1] 493	41	32	27
SPB	[1] 520	33	35	32
SPPB	[1] 498	39	33	28
SP 16 HVO	[1] 504	40	32	28
SP 8 lard	[1] 470	50	19	31
SP 16 lard	[1] 503	40	32	28
SP 8 butter	470	50	19	31
SP 16 butter	503	40	32	28
SPa 16 HVO	506	35	31	33
SPb 8 HVO	468	44	19	36
SPEW	511	37	30	33
SPEY	523	38	33	29
SPW 8 HVO	460	52	18	30
E100	684	2	59	39
Y100	739	3	74	23
Range for diets:				
High	739	52	74	39
Low	399	2	14	23

[1] Analyzed values; all other values were calculated by using 5.65 Calories per gram for protein, 4.00 Calories per gram for carbohydrates (chiefly sucrose), and 9.3 Calories per gram for fat.

Body Weight and Longevity as Influenced by Diet

Body weight, intake, and age (stock, SP 8 HVO, and SPE diets)

WEIGHT GAIN IN RELATION TO FOOD INTAKE (SP 8 HVO AND SPE DIETS).—Records of food intake and weight throughout life were maintained for 44 rats fed SP 8 HVO diet and for 38 rats fed SPE diet, and included 4 separate experimental groups of animals allocated for longevity studies on each of these diets. Data for individual groups as well as average values for all animals are summarized in appendix tables 79 and 80. Figure 1 represents graphically the average food intake and gain in weight of these rats from weaning until 300 days of age, a period relatively uncomplicated by the occurrence of excessive weight loss or death. The general pattern of food intake and weight gain was similar for both diets, although young rats fed SP 8 HVO diet tended to gain more slowly than did those fed SPE diet. From the second to the sixth week on the experimental diets, the animals progressively increased their food intake and maintained a relatively constant average rate of gain of approximately 40 grams weekly on SP 8 HVO diet and of 45 grams on SPE diet. The rats were still continuing to gain weight at 300 days of age, although their intake was relatively constant after the sixth week.

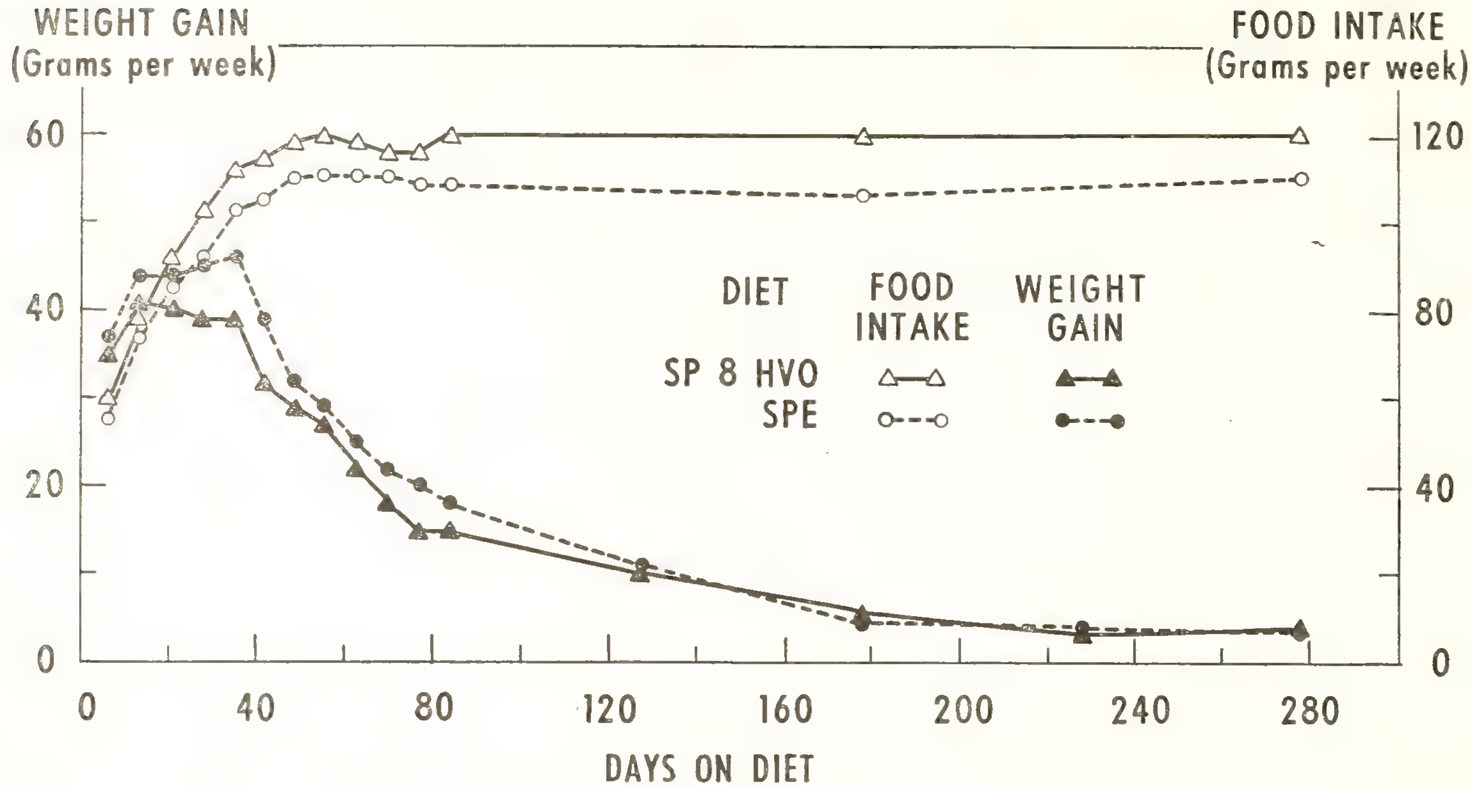

FIGURE 1. Average weight gain and food intake of rats fed SP 8 HVO and SPE diets.

BODY WEIGHT AND AGE—INDIVIDUAL RATS FED SP 8 HVO AND SPE DIETS.—Many of the rats continued to gain as long as they appeared to remain healthy, increasing their food intake whenever there was a tendency for their body weight to remain constant. This response is best illustrated in figure 2, which shows the change of weight with age of three individual animals. Curve 1 represents data for a rat that was fed SPE diet and died before he reached 400 days of age. This animal gained rapidly for 350 days, at which time a precipitous weight loss occurred in spite of a food intake averaging 17 grams per day. Curve 2 shows the continued gain for 550 days of a rat fed SPE diet and the rapid loss in weight that occurred during the 25 days before death. This animal had decreased his food intake slightly from 14 to 12 grams daily. Curve 3 represents the body weight of a rat that was fed SP 8 HVO diet and was still gaining when 800 days old. A marked decrease in food intake from 19 to 9 grams daily paralleled the decrease in body weight observed between 800 and 900 days of age, at which time the animal was obviously moribund.

AVERAGE BODY WEIGHT AND AGE (STOCK, SP 8 HVO, AND SPE DIETS).—Data for weight changes throughout life were obtained for 53 rats fed SP 8 HVO diet and for 74 fed SPE diet. The tendency for continuing increase in weight with age noted for individual rats (fig. 2) was not apparent in the average weight curves of rats fed these two diets (fig. 3). The third curve in figure 3 represents cross-sectional data available for a large group of rats from the stock colony that were sacrificed at different age intervals. Animals fed the stock diet tended to be smaller than those fed SP 8 HVO or SPE diets and showed little change in average weight after 250 days. Of these animals 52 percent weighed between 450 and 500 grams; 2 percent exceeded 600 grams. In contrast, 59 percent of the rats fed SP 8 HVO diet and 61 percent of the SPE-fed rats weighed 600 grams or more, and 21 and 18 percent, respectively, exceeded 700 grams in weight. Rats fed SPE diet appeared to have reached their maximum weight by 350 days, whereas those fed SP 8 HVO diet attained a comparable average weight between 500 and 600 days of age. The lower average weights observed for the older surviving rats fed SP 8 HVO and SPE diets seem to be due to the early death of many of the heavy rats, and will be discussed further in relation to the longevity data.

DISCUSSION.—Reports in the literature on weight changes in the rat throughout life have been chiefly for diets of natural foods suitable for raising stock animals. Changes made in the diets of stock animals based on increasing knowledge of their nutritional requirements have resulted in an appreciable increase in their size. Mendel and Hubbell (*128*) have reported a gradual increase in rate of growth of their stock ("Yale" strain) rats over a period of 25 years, with the most marked change occurring when the diet of Anderson and Smith (*9*) was introduced. This diet produced adult animals weighing about twice as much as rats on the earlier stock diets. This change was attributed to diet rather than to selective breeding, and the improved growth rate was accompanied by superior reproductive performance. Mayer (*127*), also using the "Yale" strain of rats, reported

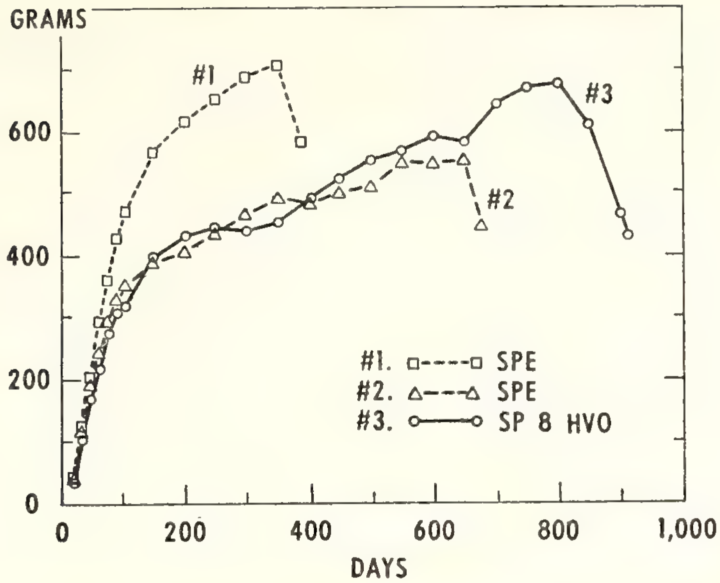

FIGURE 2.—Weight in relation to age of selected individual rats fed SP 8 HVO and SPE diets.

still more rapid weight gains for animals fed a synthetic diet.

Inherent differences in the growth potential of different strains of rats complicate comparison of the size of animals from different laboratories. Mature animals from the stock colony maintained in this laboratory appear to weigh as much as or more than most stock rats of comparable age from other laboratories. Their average maximum weight was slightly less than the 522 grams reported for the rapid-growth-producing diet of Anderson and Smith (*9*). Rats fed the semipurified diet reached weights comparable to those observed by Mayer (*127*) using a synthetic diet.

Numerous equations have been suggested to represent changes of weight with age, and several investigators (*44*, *53*, *78*) provided evidence for the usefulness of the equation proposed by Zucker, Hall, Young, and Zucker (*194*) for evaluating rat growth and relative efficiency of various experimental diets. These authors proposed an empirical formula for expressing growth which defines K, a growth intensity factor, and A, an inherent size factor. When the formula was applied to data from their laboratories as well as to data from other laboratories, these authors report that a straight line was generally observed and that neither size nor growth rate appeared to affect the growth property measured by K, the slope of the line, as long as the diets were free from growth-inhibiting factors. Dunn, Murphy, and Rockland (*53*), however, observed a change in the

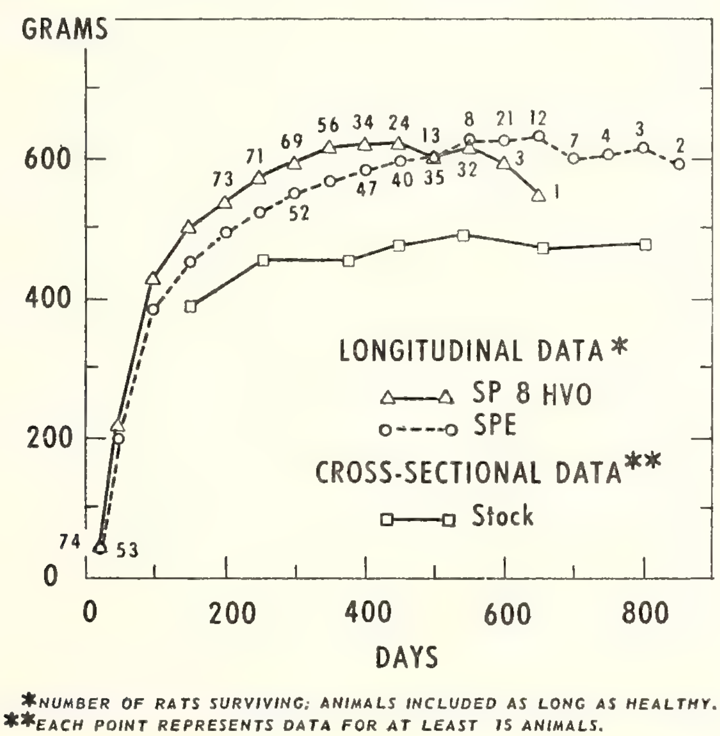

FIGURE 3.—Average weight in relation to age of rats fed stock, SP 8 HVO, and SPE diets.

slope of the line at about 14 weeks of age for rats fed the rapid-growth-producing Anderson-Smith diet, and suggested that this deflection may be related to the beginning of a normal adult period. Copping, Crowe, and Pond (*44*) observed a deflection at 11 weeks on two of the eight diets that they investigated, and suggested that this deflection may be due to the rapid early growth of rats on these two diets that contained more than adequate amounts of protein.

When Zucker's formula was applied to the data here reported for rats on SP 8 HVO and SPE diets, a straight-line relationship was found to hold reasonably well until the rats reached 15 weeks of age. During this period no deflection was apparent that would indicate any nutritional deficiencies. The K values of 3.8 for SP 8 HVO and of 4.0 for SPE diet were similar to the 3.8 value reported by Zucker for male rats. The change in the slope of the line after 15 weeks provided further evidence that the age range over which this formula applies may be limited when very rapid growth occurs in the young rat.

Everitt (*58*) and Berg and Harmison (*20*), reporting data relating body weight to age throughout life, observed a rapid period of growth followed by a plateau similar to the results reported here (fig. 3) for rats fed SP 8 HVO and SPE diets. The subsequent decline in body weight which these authors observed was also noted for the majority of rats fed SP 8 HVO and SPE diets, as seen in figure 2 in the examples for individual rats. Although there is general agreement that such weight loss frequently occurs before death, there still seems to be some question as to whether or not weight loss is a necessary accompaniment of the aging processes. Everitt (*58*) reported an average decrease in weight from 381 to 249 grams during the last 200 days of life of 68 male rats. Eighty percent of the animals had lung abscesses, but their loss in weight was similar to that in a comparable number of rats with healthy lungs. No data were reported on the incidence of other lesions except for three tumors. According to Everitt and Webb (*59*), this weight loss may be due to disease or to senescence.

Results obtained with BHE rats in this laboratory, however, as well as those reported by Berg and Harmison (*20*), indicate that weight loss in older animals generally reflects some pathological condition. These authors separated the results for rats with no lesions from those with lesions. The average age of the group without lesions was 681 days. The body weight of these rats increased with age up to 522 days, with no marked difference thereafter. A progressive decrease in weight with age was observed in the older rats with lesions.

Growth and food intake of young rats (all diets)

A pattern of weekly weight gain and food intake similar to that discussed for rats fed SP 8 HVO and SPE diets was observed for rats on the other dietary regimens under investigation. Data for a minimum of 10 rats were generally obtained with each of the experimental diets. Although there was considerable variation in the response of individual rats to any one diet, the data reported for SP 8 HVO and SPE diets summarized in appendix tables 79 and 80 indicate that average values for groups of approximately 10 rats generally agree well with those obtained for the larger number of animals.

In table 8 are summarized data on the weight gains during the first 12 weeks on each of the experimental diets, and except where indicated, the data reported have been confined to groups of littermates. Included also are data obtained on food intake in grams and in calories and on the efficiency with which these diets were used as measured by the calories-per-gram gain. Calories are reported as gross calories throughout this publication. To determine available calories from these diets, data are needed on the digestibility by the rat of the various diets under investigation, on the energy from protein that is stored in the body of the growing animal, and on the loss of energy in the urine either as protein or as incompletely oxidized products from protein. The factors 4, 9, 4, frequently used for calculating available energy from dietary protein, fat, and carbohydrate, were developed for use with man from the extensive investigations of Atwater and Bryant (*14*). Metta and Mitchell (*131*) showed that these factors are not applicable to the rat.

Modifications of semipurified diet—with selected foods.—Young rats grew well on all of the experimental diets. The most rapid growth was observed with animals fed the diets in which egg, milk, beef, or peanut butter replaced 20 to 25 percent of the semipurified diet. Weight gains were similar for all of the diets containing these foods and were consistently greater than were those observed on the semipurified diet. The difference between 16.8 Calories-per-gram gain on SP 8 HVO diet and 15.3 on SPE diet was highly significant ($P < 0.01$). Differences in digestibility of these two diets do not explain the differences observed in food utilization. Marshall and Hildebrand (*125*) recently reported that BHE rats were able to digest SP 8 HVO and SPE diets equally well. No significant differences were noted in the utilization of the diets containing egg, milk, beef, or peanut butter.

SPE diet with purified nutrients.—No change in rate of growth or in calories-per-gram gain was observed as the result of adding the various nutrient supplements to SPE diet.

Kind and level of fat.—Data for the group of diets containing different kinds and levels of fat were limited, and the calorie values of the diets were less accurately established than those for the semipurified diet or for the SP diets containing egg, milk, beef, or peanut butter. There was a consistent trend for rats fed diets containing 8 percent fat to grow more slowly than those fed the

diets containing the same fat at the 16-percent level. Rats fed the diet containing butter tended to be smaller than those fed comparable levels of HVO or lard. The diet containing 16 percent lard was the only one used with an efficiency comparable to that seen for the diets containing egg, milk, beef, or peanut butter. The range of values observed was wide, and more data on this group of diets are needed to establish the significance of these trends.

Level of protein and fat.—When diets contained 30 percent protein as casein and lactalbumin (SPb 8 HVO and SPa 16 HVO), growth rate and calorie-per-gram gain were similar to the results obtained for comparable levels of fat when the diet contained 25 percent protein (SP 8 HVO and SP 16 HVO).

Egg and egg components.—Rats fed a diet (SPEW) in which egg white replaced 30 percent of the protein in SPa 16 HVO diet tended to be smaller. Growth response and utilization of diets in which egg yolk replaced approximately 50 percent of the protein and approximately 80 percent of the fat in SP 16 HVO were similar to the results for rats on SPE diet. Rats fed a diet (SPW) in which egg white replaced all of the casein and lactalbumin in the semipurified diet were small. No data were obtained on the calories-per-gram gain for this diet. Evaluation of growth on and utilization of diets of 100 percent whole egg or egg yolk was complicated by the tendency to frequent diarrhea in the rats fed these diets. Food consumption and caloric intake of these two high-fat diets were low, and rate of growth was correspondingly reduced.

Wistar rats fed SP 8 HVO and SPE diets.—Wistar rats fed SP 8 HVO and SPE diets ate less and grew at a slower rate than did BHE rats fed comparable diets. Wistar rats seemed to use both diets more efficiently for growth than did comparable BHE rats, but the differences observed may be due in whole or in part to the smaller weight that was being maintained by the Wistar rats during this period of rapid growth. However, both Wistar and BHE rats tended to grow more rapidly and to use their food more efficiently when fed SPE diet than when fed SP 8 HVO diet.

Discussion.—Although the levels of fat in the diets investigated were not considered excessive, there appeared to be a consistent trend for rats to become larger at an earlier age when the fat level was 17 to 19 percent than when it was 9 percent or less. In some instances, the increased growth rate appeared to be explained by differences in intake alone; in others, a decrease in calories-per-gram gain—that is, more efficient utilization of the diet—accompanied the increased growth rate observed.

Deuel (*48*) in reviewing the subject of dietary fat and growth, concluded that the preponderance of evidence favors the hypothesis that in the rat greater increases in weight and improved efficiencies of the diet are associated with increased consumption of fat. In studying the associative dynamic effects of protein, carbohydrate, and fat, Forbes and Swift (*64*) showed that fat is particularly effective in reducing the specific dynamic effects of diets.

Reports in the literature on the response of the rat to fat have dealt chiefly with the influence of different kinds and levels of fat rather than with fat-containing foods such as egg, milk, beef, or peanut butter. Lard has been most frequently used in investigations dealing with the response to dietary fat. In weanling rats fed for a period of from 8 to 10 weeks, there has been a tendency for weight gain to correlate with the level of this fat. The differences reported have usually been small and in some investigations have not proved statistically significant.

Hoagland and Snider (*93*) found weight gains per 100 Calories to be approximately the same for diets containing 15, 30, or 54 percent lard, but at these levels the gains were significantly greater than those observed with 5 percent of this fat. Hoagland, Snider, and Swift (*94*) found that the differences were not significant when isocaloric amounts of diets containing 5, 10.98, or 18.27 percent lard were fed. Forbes, Swift, Elliot, and James (*65*) reported weight gains to correlate with level of fat when rats were fed isocaloric quantities of diets containing 2, 5, 10, or 30 percent fat. Two percent corn oil was present in all of the diets, and the additional fat was lard. The largest difference observed was that between the diets containing 2 percent fat and those containing 5 percent fat. Forbes, Swift, James, and others (*67*) reported a similar experiment using large increases in the intake of 10 of the vitamins, and again demonstrated that fat confers efficiency of utilization of food energy for the growing albino rat; however, the small increments in weight gain observed as the level of fat increased from 2 to 30 percent were not statistically significant.

Hoagland and Snider (*93*) compared the relative efficiency of lard and hydrogenated cottonseed oil as dietary fats. At all except the lowest level tested (5 percent), lard proved significantly more efficient in promoting growth than did hydrogenated cottonseed oil. The diet containing 15 percent hydrogenated cottonseed oil appeared somewhat more efficient than the diet containing 5 percent of this fat; at still higher levels the response was similar to that observed with diets containing 15 percent fat. Marshall, Hildebrand, Dupont, and Womack (*126*) fed ad libitum diets containing 15 percent hydrogenated vegetable oil or lard. Weight gain per 100 Calories during the period of rapid growth was slightly more on the diet containing lard, but the differences were small and not statistically significant.

According to Barki, Collins, Elvehjem, and Hart (*17*), conclusions with regard to the growth-promoting properties of fats may be misleading if comparisons are confined to one level of fat. These

TABLE 8.—*Growth, food intake, and calories per gram of gain in young rats fed various diets*

Strain and diet	Rats	Weaning weight		Weight gain first 12 weeks		Food intake first 12 weeks		Gross calorie intake–12 weeks		Calories-per-gram gain	
		Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
BHE rats	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Calories*	*Calories*		
All fed—											
SP 8 HVO	54	46	38–62	360	273–506	1, 290	1, 040–1, 600	6, 050	4, 870–7, 540	16. 8	14. 9–19. 2
SPE	48	46	39–62	400	310–471	1, 180	960–1, 380	6, 140	5, 020–7, 190	15. 3	13. 4–16. 8
Littermates fed—											
SP 8 HVO	24	47	38–62	373	301–506	1, 350	1, 110–1, 600	6, 330	5, 200–7, 540	17. 0	14. 9–18. 7
SPE	24	47	39–57	404	310–471	1, 190	960–1, 380	6, 200	5, 020–7, 190	15. 3	14. 6–16. 3
SPM	24	47	37–59	405	331–505	1, 280	1, 140–1, 500	6, 290	5, 630–7, 400	15. 5	14. 4–17. 0
SPB	24	47	39–56	400	347–463	1, 180	1, 000–1, 390	6, 140	5, 230–7, 240	15. 4	14. 0–16. 5
SPPB	24	47	39–60	412	310–478	1, 230	1, 050–1, 420	6, 120	5, 220–7, 070	14. 9	14. 1–16. 8
Littermates fed—											
SPE supplemented with—											
None	10	45	40–62	420	362–457	1, 200	1, 090–1, 310	6, 260	5, 670–6, 810	14. 9	13. 4–15. 7
Choline, 0.5%	10	46	33–58	442	387–552	1, 270	1, 090–1, 490	6, 600	5, 670–8, 650	14. 9	13. 6–15. 6
B_{12}, 0.01 mg./100 gm	10	45	40–60	428	389–484	1, 240	1, 140–1, 410	6, 460	5, 930–7, 330	15. 1	13. 8–16. 5
Choline, 0.5% + B_{12}, 0.01 mg./100 gm	10	45	39–57	429	361–513	1, 260	1, 100–1, 480	6, 540	5, 690–7, 680	15. 2	14. 5–16. 0
Littermates fed—											
SPE supplemented with—											
None	10	48	37–61	386	328–454	1, 150	1, 000–1, 370	5, 980	5, 200–7, 120	15. 5	14. 5–16. 1
Choline, 0.5%	10	49	42–65	408	364–443	1, 180	1, 060–1, 240	6, 150	5, 510–6, 450	15. 1	13. 4–15. 7
B_6, 0.5 mg./100 gm	10	49	38–61	405	365–451	1, 190	1, 010–1, 350	6, 170	5, 240–7, 000	15. 2	12. 5–16. 5
Choline, 0.5% + B_6, 0.5 mg./100 gm	10	48	37–62	399	355–497	1, 180	1, 100–1, 380	6, 120	5, 710–7, 200	15. 3	14. 5–16. 4
Choline, 0.5% + B_6, 0.5 mg./100 gm. + B_{12}, 0.01 mg./100 gm	10	48	38–61	408	331–460	1, 200	1, 070–1, 320	6, 260	5, 570–6, 860	15. 3	14. 5–16. 8
Littermates fed—											
SPE supplemented with—											
None	10	47	39–56	395	331–456	—	—	—	—	—	—
Cholesterol, 0.46%	10	48	37–60	405	291–519	—	—	—	—	—	—
Cholesterol, 1.38%	10	47	37–54	401	316–442	—	—	—	—	—	—
Ascorbic acid, 0.2%	10	47	38–55	401	324–470	—	—	—	—	—	—
Ascorbic acid, 0.2% + cholesterol, 0.46%	10	47	37–55	400	341–458	—	—	—	—	—	—
Littermates fed—											
SP 8 HVO	10	44	38–50	350	273–407	1, 240	1, 040–1, 390	5, 810	4, 870–6, 530	16. 7	16. 0–17. 9
SP 16 HVO	10	44	38–50	372	305–421	1, 193	1, 020–1, 310	6, 010	5, 130–6, 620	16. 3	15. 0–17. 7
SP 8 lard	10	45	38–48	356	306–419	1, 260	1, 050–1, 540	5, 930	4, 940–7, 260	16. 6	15. 6–18. 2
SP 16 lard	10	44	39–54	377	338–427	1, 172	1, 010–1, 390	5, 890	5, 100–7, 000	15. 6	15. 1–16. 4
SP 8 butter	10	44	38–52	337	289–394	1, 250	1, 110–1, 490	5, 880	5, 230–7, 010	17. 4	16. 2–18. 1
SP 16 butter	10	42	38–51	354	285–427	1, 200	1, 000–1, 450	6, 030	5, 000–7, 310	17. 0	16. 0–18. 0
Littermates fed—											
SP 8 HVO	10	46	39–60	345	303–396	1, 240	1, 080–1, 430	5, 830	5, 070–6, 730	16. 9	15. 7–19. 8
SPa 16 HVO	10	46	41–56	385	324–479	1, 210	1, 030–1, 440	6, 140	5, 230–7, 310	15. 9	15. 2–17. 1
SPb 8 HVO	10	47	39–60	358	295–453	1, 275	1, 100–1, 560	5, 970	5, 160–7, 320	16. 7	15. 0–19. 2

Littermates fed—											
SPE (fresh egg)	10	45	39–52	377	314–469	1, 160	990–1, 340	6, 030	5, 130–6, 970	16. 0	14. 5–18. 2
SPEW	10	44	39–51	352	283–408	1, 150	970–1, 290	5, 860	4, 970–6, 620	16. 6	15. 1–18. 2
SPEY	10	45	38–51	400	324–474	1, 190	990–1, 370	6, 210	5, 160–6, 960	15. 6	14. 5–16. 6
E100	10	45	39–53	296	243–383	780	700– 970	5, 300	4, 750–6, 620	18. 0	16. 7–19. 6
Littermates fed—											
SPE	5	50	41–59	353	309–405	1, 120	1, 010–1, 200	5, 820	5, 270–6, 260	16. 6	15. 1–18. 8
Y100	5	47	39–58	248	185–306	600	510– 660	4, 400	3, 800–4, 840	17. 8	15. 2–20. 9
Y97+salt mixture, 3.0%	5	46	39–53	303	265–334	710	650– 760	5, 100	4, 670–5, 450	16. 8	15. 5–18. 7
Not littermates fed—											
SPW	10	58	45–71	280	212–354	—	—	—	—	—	—
E100	15	43	39–49	315	273–355	804	700– 870	5, 500	4, 780–5, 950	17. 5	15. 5–20. 6
Y100	10	42	38–56	292	231–341	637	550– 730	4, 710	4, 050–5, 400	16. 1	15. 0–17. 5
Wistar rats											
Littermates fed—											
SP 8 HVO	10	48	39–55	324	266–380	1, 100	890–1, 320	5, 170	4, 170–6, 230	16. 0	14. 4–16. 9
SPE	10	48	37–53	364	326–429	1, 020	920–1, 100	5, 300	4, 780–5, 760	14. 6	13. 4–15. 5

investigators reported that early growth of young pair-fed rats was consistently less when the diet contained 10 or 28 percent butterfat than when it contained a comparable level of corn oil. When the level of butterfat was increased to 35 percent, rats were as large as or larger than those on diets containing 10, 28, or 35 percent corn, soybean, or coconut oil. More efficient utilization of the diets was observed as the level of butter was increased; no such relation was noted for corn oil. Pair feeding and ad libitum feeding gave similar results.

Hoagland and Snider (*92*) observed a growth-promoting value of peanut oil (gain per 100 Calories) similar to that obtained with lard or hydrogenated cottonseed oil when the level of fat was 5 percent. At 30-percent levels, diets containing lard were used more efficiently than either hydrogenated cottonseed oil or peanut oil. Aaes-Jorgensen and Dam (*1*, *2*) included peanut oil in many of their investigations, and in general found similar growth-promoting qualities for lard and for peanut oil. The increase in growth rate that resulted when the fat level was increased from 7 to 28 percent was not due to increased caloric intake.

Body weight in relation to age and diet in adult rats

SP 8 HVO, SPE, SPM, SPB, and SPPB diets.—Differences were observed in the rate at which adult rats gained weight and in the maximum weight eventually attained, even when diets produced a similar growth response in young animals. In table 9 are summarized data showing the influence of diet on body weight at different ages. Included also for ready reference are the more extensive data already considered for SP 8 HVO and SPE diets. Adult rats fed the diets containing milk, beef, or peanut butter continued to gain throughout their healthy lifespan and were consistently heavier than rats of similar age fed the semipurified diet. They also tended to become heavier than rats fed the SPE diet. The older surviving rats fed SPM or SPPB diet tended to be larger than those of comparable age fed SPB diet. Differences in caloric intake did not account for some of the weight differences observed. For example, between 500 and 600 days of age the rats fed SPM diet gained, on the average, 70 grams, whereas those fed SPB diet gained 22 grams. The intakes for rats fed these two diets were similar—9,370 and 9,340 Calories respectively. Additional data on the caloric intakes of these rats at different ages are included in appendix table 80.

Kind and level of fat.—Differences in the weights of rats fed diets containing HVO, lard, or butter seemed to be attributable chiefly to differences in caloric intake except for rats fed 16 percent lard. At 300 days of age on a similar caloric intake, the average weight of rats fed semipurified diet was 60 grams less than that for the corresponding littermates fed SP 16 lard.

Level of protein and fat.—The caloric consumption of rats fed SPa 16 HVO and SPb 8 HVO was approximately 5 percent more than that of rats fed the semipurified diet, and accounted for the tendency to larger animals on these two diets.

Egg and egg components.—No appreciable change in the weight curve of adult rats resulted from replacing 30 percent of the protein of SPa 16 HVO diet with egg white (SPEW). When egg yolk supplied all of the fat in the diet (SPEY), weight changes for the adult, like those for the young rat, resembled those observed with SPE diet. Rats fed 100 percent egg (E100) remained relatively small but did eventually reach weights similar to those of stock rats. On SPW diet, rats tended to be small throughout life although the weight of the older rats was somewhat greater than that of the rats fed E100 diet. On 100 percent egg yolk, food consumption increased with age, so that weights of rats surviving 400 days were comparable to those of SP 8 HVO rats.

Wistar rats fed SP 8 HVO and SPE diets.—Wistar rats fed SP 8 HVO and SPE diets showed a somewhat different age-weight relationship than did BHE rats. On the semipurified diet, Wistar rats were consistently smaller than BHE rats of comparable age; on SPE diet, Wistar rats eventually became much heavier.

Discussion.—Reports in the literature on diet in relation to weight throughout the life of the adult rat are limited and, as with the growing rat, deal chiefly with the level or kind of dietary fat. No reports have been located dealing with protein-fat-containing foods such as have been included in this publication. In general, the results obtained in this laboratory and elsewhere provide considerable evidence that differences in weight gain are not necessarily associated with differences in caloric intake.

With mature rats 205 to 212 days old, Forbes, Swift, Elliott, and James (*66*) and Forbes, Swift, Thacker, and others (*68*) observed that the energy expense of utilization of isocaloric diets decreased as the level of dietary fat (chiefly lard) increased from 2 to 30 percent. A reduction in heat from the catabolism of carbohydrates and from fat synthesis was responsible for the resulting economy of utilization of food energy as the level of fat increased. French, Ingram, Uram, and others (*69*) reported that by the time rats were 28 weeks old on a diet in which 20 percent of the stock diet was replaced with corn oil, their weight exceeded significantly that of animals fed a diet in which 20 percent of the stock diet was replaced with sucrose. Lundback and Stevenson (*119*) obtained a gain of 0.6 gram per day for adult rats fed a diet containing 60 percent fat chiefly as lard, in contrast to no weight gain for rats on a comparable caloric intake of a diet containing 71 percent sucrose.

TABLE 9.—*Body weight of rats at different ages fed various diets*

Strain and diet	Rats	Weaning weight	Weight at[1]—								
			50 days	100 days	200 days	300 days	400 days	500 days	600 days	700 days	800 days
BHE rats											
All fed—	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
SP 8 HVO	53	46	200	384	496	554 (52)	585 (47)	603 (35)	629 (21)	601 (7)	618 (3)
SPE	74	46	222	430	542 (73)	597 (69)	624 (34)	603 (13)	595 (3)		
Littermates fed—											
SP 8 HVO	14	46	209	399	494	551 (13)	584 (10)	566 (7)	542 (3)	574 (2)	593 (1)
SPE	14	46	221	438	547	598	574 (4)	515 (2)	550 (1)		
SPM	14	46	209	429	556 (13)	622	662 (11)	659 (7)	732 (6)	775 (6)	
SPB	14	46	213	430	538	609	632 (10)	665 (5)	667 (4)	726 (2)	
SPPB	14	46	220	442	568 (13)	618 (12)	653 (9)	664 (6)	728 (5)	810 (2)	
Littermates fed—											
SP 8 HVO	10	44	197	380	521	581	569 (9)	568 (6)	572 (4)	588 (3)	
SP 16 HVO	10	44	205	400	535	617	626 (9)	632 (7)	653 (5)	705 (3)	
SP 8 lard	10	45	207	385	516	604	599 (6)	632 (4)	664 (4)	714 (1)	
SP 16 lard	10	44	204	403	551	640	610 (7)	639 (5)	652 (2)	750 (1)	
SP 8 butter	10	44	189	368	504	598 (9)	602 (8)	605 (7)	629 (5)	551 (1)	
SP 16 butter	10	42	192	384	519	595	590 (6)	537 (3)	587 (3)		
Littermates fed—											
SP 8 HVO	10	46	201	378	487	553	607	620 (7)	762 (4)	607 (1)	
SPa 16 HVO	10	46	207	417	528 (9)	606	632 (8)	688 (3)	666 (2)	727 (1)	
SPb 8 HVO	10	47	206	389	507	576	624 (9)	628 (7)	684 (5)	694 (3)	
Littermates fed—											
SPE (fresh egg)	10	45	215	413	554	617	626 (7)	686 (4)			
SPEW	10	44	204	388	541	599	612 (8)	661 (7)	607 (4)	673 (2)	
SPEY	10	45	216	434	584	636	629 (6)	682 (3)			
E100	10	45	180	332	443	494	472 (8)	471 (6)	506 (2)		
Not littermates fed—											
SPW	7	59	190	317	429	513	542	553 (6)			
Y100	10	42	127	315	469 (9)	510 (7)	585 (4)	601 (1)			
Wistar rats											
Littermates fed—											
SP 8 HVO	10	48	193	363	455	472	493	518 (8)	548 (7)	564 (5)	599 (2)
SPE	10	48	213	398	515	550 (8)	587 (7)	665	688 (6)	706	771 (3)

[1] Data are for rats maintained on the diets indicated throughout life. Numbers in parentheses indicate the number of rats still maintaining weight at the ages indicated.

Calories for maintenance of body weight

BHE rats.—Although many of the animals tended to gain weight throughout most of their lifespan regardless of diet, there were often 50- to 100-day intervals when weight remained relatively constant. The caloric intake during these intervals served as a basis for calculating calories required for maintenance. The results available are recorded in table 10. The values were obtained for rats varying considerably in weight, but in general included data for rats on each diet with a similar weight range. On the basis of the more extensive data obtained for rats fed SP 8 HVO diet, the calories required for maintenance appeared to decrease with increasing age and/or body weight. With rats fed SPE diet, however, no such relationship appeared to exist. The tendency for heavy rats on those diets with the lowest maintenance requirement suggests that there may be a real difference in the way that these diets are utilized. The differences observed in the average calorie requirements with diet, however, were relatively small considering the wide range of values observed for individual rats. More data under controlled conditions of intake and activity are needed to establish the significance of these trends.

Table 10.—*Calories per gram of body weight per week for maintenance of adult rats fed various diets*

Strain and diet	Rats	Calories per gram of body weight	
		Average	Range
BHE rats	*Number*		
SP 8 HVO	26	0. 98	0. 82–1. 08
SPE	24	. 95	. 73–1. 14
SPM	8	. 90	. 76– . 99
SPB	4	. 96	. 93– . 98
SPPB	6	. 90	. 84– . 95
SP 16 HVO	8	. 95	. 85–1. 13
SP 8 lard	8	. 92	. 80–1. 05
SP 16 lard	6	. 89	. 79– . 95
SP 8 butter	8	. 92	. 89– . 99
SP 16 butter	8	. 91	. 79–1. 04
Wistar rats			
SP 8 HVO	7	. 87	. 83– . 96
SPE	5	. 79	. 73– . 85

Wistar rats.—The lower calorie requirement for maintenance of the Wistar rats fed SP 8 HVO or SPE diets when compared with BHE rats parallels the greater efficiency in their use of these diets during early growth. In the young rats, the differences were due in part at least to the somewhat smaller body weight that was being maintained during this period by Wistar rats. A comparison of the weight and intake of the two strains of rats fed SPE diet (appendix table 80) shows a lower intake even by older Wistar rats during periods when the weight was as great as or greater than that of BHE rats.

Discussion.—Little information is available on the energy requirements for maintenance of the adult rat. Fixsen and Jackson (*62*) estimated the requirement of the rat at 12.0 metabolizable Calories per 100 grams per day for animals weighing 375 grams or less, and 11.5 metabolizable Calories per 100 grams for those weighing more than 375 grams. With two strains of rats, Palmer, Kennedy, Calverley, and others (*151*) demonstrated a difference in the utilization of foods for growth and in the energy requirement for maintenance. The high-efficiency strain of animals stored a larger proportion of their food energy and lost less energy as heat than did the low-efficiency strain. These authors, using 4.1 Calories per gram for protein and carbohydrate and 9.3 for fat, obtained a value of 13.2 Calories per gram per day for maintenance of the high-efficiency rats and 14.6 for the low-efficiency animals. Application of these factors to the data reported in this publication for BHE rats fed SP 8 HVO diet resulted in a value of 12.7, similar to that of the high-efficiency strain. Wistar rats appeared to be still more efficient in their utilization of this same diet for maintenance.

Maximum body weight and diet

Average weight curves provide little information with regard to the variation in the rate of gain or in the maximum weight attained by individual rats. In table 11 are summarized data on the average maximum weight and the range of values observed for rats sacrificed between 300 and 500 days of age and for those that were older than 500 days. These values include data for rats scheduled for sacrifice in the age groups indicated, as well as those from longevity studies, and thus represent a relatively larger group of animals for some of the diets than are included in table 9. Data are also summarized for rats fed the various supplemented SPE diets and for a small group of rats fed SP 8 HVO and SPE diets from parents raised on the Wistar stock diet.

Although the average maximum weights observed were generally higher, particularly for the younger group of rats, than were apparent from the average weight-age relationships seen in table 9, the results show the same general trend that has already been discussed. Rats fed SPE diet with the various supplements were similar in size to those fed the unsupplemented diet. When BHE rats were fed the stock diet on which the Wistar rats had been fed, weanling rats tended to be small and generally reached a maximum weight on SP 8 HVO or SPE diet that was less than that observed with these same diets when the young were from parents raised on the usual stock ration.

Diet and obesity.—Large rats were observed with all of the experimental diets, particularly with SPM or SPPB diet. The largest rat was a

TABLE 11.—*Maximum weight of rats fed various diets, sacrificed before or after 500 days of age*

Strain and diet	Sacrificed at 300–499 days				Sacrificed at 500 days or over			
	Rats	Average age	Weight		Rats	Average age	Weight	
			Average	Range			Average	Range
	Number	*Days*	*Grams*	*Grams*	*Number*	*Days*	*Grams*	*Grams*
BHE rats								
Stock	31	416	470	370–579	57	680	484	399–670
SP 8 HVO	52	380	602	423–771	57	636	663	514–890
Protein-fat-containing foods:								
SPE	131	392	626	456–790	65	575	645	505–792
SPM	20	408	684	575–791	21	649	753	85–1,020
SPB	19	410	615	486–800	23	618	690	565–902
SPPB	26	394	680	510–976	28	581	715	610–908
SPE supplemented with—								
Choline, 0.5%	16	419	659	548–756	8	584	670	583–790
B_{12}, 0.01 mg./100 gm	6	412	630	573–713	3	566	713	666–780
Choline, 0.5%+B_{12}, 0.01 mg./100 gm	6	398	648	511–764	3	562	730	668–767
B_6, 0.5 mg./100 gm	4	408	664	565–790	4	570	666	645–713
Choline, 0.5%+B_6, 0.5 mg./100 gm	9	412	654	590–735	1	508	623	623
Choline, 0.5%+B_{12}, 0.01 mg./100 gm.+B_6, 0.5 mg./100 gm	8	408	661	605–723	2	522	624	564, 685
Cholesterol, 0.46%	7	416	648	568–856	3	552	660	544–820
Cholesterol, 1.38%	6	402	644	536–765	2	578	665	627, 703
Ascorbic acid, 0.2%	5	377	630	510–740	3	535	596	500–769
Ascorbic acid, 0.2%+cholesterol, 0.46%	8	394	645	551–781	2	531	689	600, 778
Fat, 8% or 16%:								
SP 16 HVO	29	409	663	565–780	27	611	709	593–875
SP 8 lard	6	429	613	550–684	4	673	676	627–722
SP 16 lard	4	416	686	630–766	6	652	675	599–797
SP 8 butter	2	394	600	597, 602	7	662	645	564, 732
SP 16 butter	6	409	627	593–662	4	644	624	528–700
SP 8 HVO with protein or fat to level of egg:								
SPa 16 HVO	4	454	651	571–736	15	696	725	555–982
SPb 8 HVO	3	456	635	501–845	7	689	674	524–870
Egg and egg fractions:								
SPE (fresh egg)	6	429	633	566–729	4	562	687	566–788
SPW	1	459	524	524	6	550	574	440–668
SPEW	3	407	598	497–651	7	652	692	601–791
SPEY	5	420	668	582–744	5	560	666	590–763
E100	8	451	541	442–668	17	555	541	424–698
Y100	14	402	522	448–698	6	539	589	532–646
Y97+salt mixture, 3%	5	430	555	518–595	---	---	---	---
Diet reversal:								
Stock throughout life	1	420	537	537	2	675	612	528, 697
SPE throughout life	2	396	554	530, 578	1	629	825	825
Stock changed to SPE at 250 days	0	---	---	---	4	686	694	608–791
SPE changed to stock at 250 days	1	384	535	535	3	705	680	643–709
Wistar rats								
SP 8 HVO	1	446	445	445	9	772	576	475–673
SPE	2	355	538	519, 673	7	791	758	588–970
BHE parents fed Wistar stock diet								
BHE young fed—								
SP 8 HVO	0	---	---	---	9	759	561	482–684
SPE	3	358	533	446–583	5	592	565	510–616

720-day-old animal fed SPM diet that weighed 1,020 grams. Among the rats 500 days and older that were fed this diet, four reached weights exceeding 900 grams. On SPPB diet, one rat reached a maximum weight of 976 grams by 341 days, and three additional animals that were less than 500 days old reached maximum weights exceeding 800 grams. No extremely large Wistar rats were obtained with the semipurified diet, but with SPE diet one rat weighed 970 grams at 750 days of age, exceeding by 178 grams the largest BHE rat fed this diet.

No quantitative data on body composition were obtained for the studies reported in this

bulletin, but animals that were obviously obese were obtained with several of the experimental diets. Marshall, Hildebrand, Dupont, and Womack (*126*) observed for adult rats an apparent difference in the conversion to body fat of calories from different diets, even on comparable calorie intake. Apparent differences in the utilization of calories by the adult rat fed some of the diets such as SPM or SPPB may be related to the extent of the conversion of calories from these diets to body fat.

Discussion.—Mickelsen, Takahashi, and Craig (*132*) observed exceedingly obese rats when the Osborne and Mendel strain of animals were fed ad libitum a diet containing 60 percent Crisco. The largest animal weighed 1,655 grams. Sprague Dawley rats or NIH black rats fed these high-fat diets became heavier than stock animals but not so heavy as the Osborne and Mendel strain. Obese rats approaching 1,000 grams in weight were also observed when the Osborne and Mendel strain were fed the authors' "best" low-fat regimen for a period of 60 weeks. The weight curves of these rats fed the low-fat diet (3 percent) were similar to those already discussed for BHE rats fed many of the experimental diets. A reduction in growth rate occurred at about 15 weeks, with many of the rats continuing to gain slowly throughout the 60-week experimental period. Some rats showed a spurt in body weight gain at the 20th or 30th week. This "best" low-fat diet of Mickelsen, Takahashi, and Craig (*132*) was a relatively simple diet with casein as the chief source of protein and with a protein level of 25 percent. Sucrose was the carbohydrate in this diet, amounting to 66 percent.

The sucrose content of the semipurified diet and its various modifications discussed in this publication was high (39 to 52 percent) and may be responsible for the ready acceptance of these diets by BHE rats. Food intakes frequently were as much as 19 or 20 grams daily even at a relatively early age. The high digestibility and correspondingly low fecal bulk observed by Marshall, Hildebrand, Dupont, and Womack (*126*) with similar diets make it possible for rats to consume excessive amounts of these diets without apparent digestive disturbances. In general, the results obtained with BHE rats provide further evidence that obesity in normal male rats may be produced by diet and that rats may overeat voluntarily if supplied with diets that are sufficiently acceptable and that can be consumed in relatively large amounts. The failure to obtain equally large rats when the stock diet was fed was probably due to a limitation in the amount of this diet that could be consumed because of the large fecal bulk and the poor digestibility observed with this diet (*126*).

Longevity

In table 12 are summarized data dealing with the survival of rats on all of the experimental regimens under investigation. In addition to the average results for the more extensive investigations with SP 8 HVO and SPE diets, data are also presented for individual series to permit a direct comparison of the response of littermates to those diets for which only limited information is available.

SP 8 HVO diet.—A group of 53 rats representing five experimental series on the semipurified diet provides data on the influence of this diet on longevity. The average age of the animals at death was 629 days, with 50 percent dead by the end of 616 days. Fourteen rats survived more than 700 days. Figure 4 shows the percentage of rats that died within different age intervals on this diet. The highest death rate occurred between 600 and 700 days.

SPE diet.—Similar data were obtained for 85 rats representing eight experimental series on SPE diet (table 12). The lifespan of these rats was considerably shorter than that observed with the semipurified diet; average age at death was 464 days, with 50 percent dead by 449 days. Only one of the 85 rats survived more than 700 days. As shown in figure 4, the maximum death rate for these rats occurred between 400 and 500 days of age.

Protein-fat-containing foods.—Two series of rats were fed the diets containing milk, beef, or peanut butter. In addition, comparative data were obtained on the response of littermates to SP 8 HVO or SPE diets. The results for the 21 littermates fed SP 8 HVO or SPE diets were, in general, similar to those already discussed for the larger number of animals fed these diets. The shortest lifespan was observed for animals fed SPE diet. Rats fed the SPPB diet also tended to die at an early age. Although the average age at death was approximately the same—about 580 days—for rats fed SP 8 HVO, SPM, or SPB diets, the number of rats dying within comparable age intervals was not the same.

Figure 5 presents data for SPM, SPB, and SPPB diets similar to that seen in figure 4 for SP 8 HVO and SPE diets. On SPM diet there were several early deaths but 8 of the 21 rats survived more than 700 days, in contrast to only 3 of the littermates fed SP 8 HVO diet. On SPB diet only 1 rat died before reaching 400 days of age; the maximum death rate occurred between 600 and 700 days; 4 rats survived beyond 700 days. On SPPB diet the maximum death rate occurred at a somewhat earlier age, between 500 and 600 days, with 5 of the 21 rats dead before 400 days of age.

SPE diet with selected supplements.—Rats fed SPE diet supplemented with choline, vitamin B_6, or vitamin B_{12}, alone or in combination, were generally as short lived as on SPE diet alone, and there was no evidence that any of the supplements investigated had a measurable effect on the longevity of these animals. The addition of cholesterol or ascorbic acid also seemed to exert little influence on the length of life of rats fed SPE diet.

TABLE 12.—*Age at death and mortality rate of rats fed various diets*

Strain and diet	Rats	Age at death: Average	Age at death: 50 percent died by—	Age at death: Oldest rat	Rats dying before—: 400 days	500 days	600 days	700 days	800 days	900 days	1,000 days	1,100 days
BHE rats	*Number*	*Days*	*Days*	*Days*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
All fed—												
SP 8 HVO	53	629	616	917	2	17	43	73	86	96	100	------
SPE	85	464	449	705	28	64	88	99	100	------	------	------
Littermates fed—												
SP 8 HVO	21	579	574	889	5	24	57	86	91	100	------	------
SPE	21	467	419	705	38	57	76	95	100	------	------	------
SPM	21	580	578	903	19	43	57	62	86	95	100	------
SPB	21	589	603	852	5	29	43	81	95	100	------	------
SPPB	21	525	541	722	23	37	66	85	100	------	------	------
Littermates fed— SPE supplemented with—												
None	8	444	435	581	25	62	100	------	------	------	------	------
Choline, 0.5%	8	441	414	551	38	88	100	------	------	------	------	------
B_{12}, 0.01 mg./100 gm	8	475	431	632	25	62	88	100	------	------	------	------
Choline, 0.5% + B_{12}, 0.01 mg./100 gm	8	423	400	642	38	75	88	100	------	------	------	------
Littermates fed— SPE supplemented with—												
None	9	458	426	626	33	78	89	100	------	------	------	------
Choline, 0.5%	9	432	410	581	22	89	100	------	------	------	------	------
B_6, 0.5 mg./100 gm	9	464	423	625	33	56	89	100	------	------	------	------
Choline, 0.5% + B_6, 0.5 mg./100 gm	9	427	418	508	22	79	100	------	------	------	------	------
Choline, 0.5% + B_6, 0.5 mg./100 gm. + B_{12}, 0.01 mg./100 gm	9	443	438	527	22	78	100	------	------	------	------	------
Littermates fed— SPE supplemented with—												
None	8	415	375	597	50	88	100	------	------	------	------	------
Cholesterol, 0.46%	8	477	474	578	25	62	100	------	------	------	------	------
Cholesterol, 1.38%	8	410	406	574	38	88	100	------	------	------	------	------
Ascorbic acid, 0.2%	8	405	361	533	50	75	100	------	------	------	------	------
Ascorbic acid, 0.2% + cholesterol, 0.46%	8	414	358	520	50	88	100	------	------	------	------	------
Littermates fed—												
SP 8 HVO	9	631	643	774	0	22	33	67	100	------	------	------
SP 16 HVO	9	618	569	775	11	11	44	67	100	------	------	------
SP 8 lard	9	524	444	706	11	67	67	89	100	------	------	------
SP 16 lard	9	553	506	776	22	44	56	78	100	------	------	------
SP 8 butter	9	602	610	774	11	22	33	78	100	------	------	------
SP 16 butter	9	514	487	707	33	56	67	78	100	------	------	------
Littermates fed—												
SP 8 HVO	16	618	614	889	6	25	44	69	94	100	------	------
SP 8 lard	16	566	518	799	12	44	56	81	100	------	------	------
Littermates fed—												
SP 8 HVO	8	676	623	917	0	0	25	75	88	88	100	------
SPa 16 HVO	8	582	522	810	0	38	62	75	88	100	------	------
SPb 8 HVO	8	593	505	813	0	38	50	88	88	100	------	------
Littermates fed—												
SP 8 HVO	18	680	653	917	0	11	28	56	78	89	100	------
SPa 16 HVO	18	654	621	893	0	17	38	61	83	100	------	------
Littermates fed—												
SPE (fresh egg)	10	482	465	604	10	60	90	100	------	------	------	------
SPEW	10	578	552	749	10	30	50	70	100	------	------	------
SPEY	10	490	441	585	10	50	100	------	------	------	------	------
E100	10	548	525	696	10	30	70	100	------	------	------	------
Not littermates fed—												
E100	25	559	522	762	8	32	64	92	100	------	------	------
Y100	19	393	381	591	58	74	100	------	------	------	------	------
Littermates fed—												
Stock	3	590	------	727	0	33	33	67	100	------	------	------
SPE	3	473	------	629	67	67	67	100	------	------	------	------
Stock changed to SPE at 250 days	4	686	------	805	0	0	25	25	75	100	------	------
SPE changed to stock at 250 days	4	624	------	767	25	25	25	50	100	------	------	------

TABLE 12.—*Age at death and mortality rate of rats fed various diets*—Continued

Strain and diet	Rats	Age at death			Rats dying before—							
		Average	50 percent died by—	Oldest rat	400 days	500 days	600 days	700 days	800 days	900 days	1,000 days	1,100 days
Wistar rats												
Littermates fed—	*Number*	*Days*	*Days*	*Days*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
SP 8 HVO	10	739	836	876	0	10	20	40	40	100		
SPE	10	654	742	888	30	30	40	40	60	100		
BHE parents fed Wistar stock diet												
Young fed—												
SP 8 HVO	9	703	755	1, 028	11	11	33	33	78	78	78	100
SPE	9	424	381	762	44	56	89	89	100			

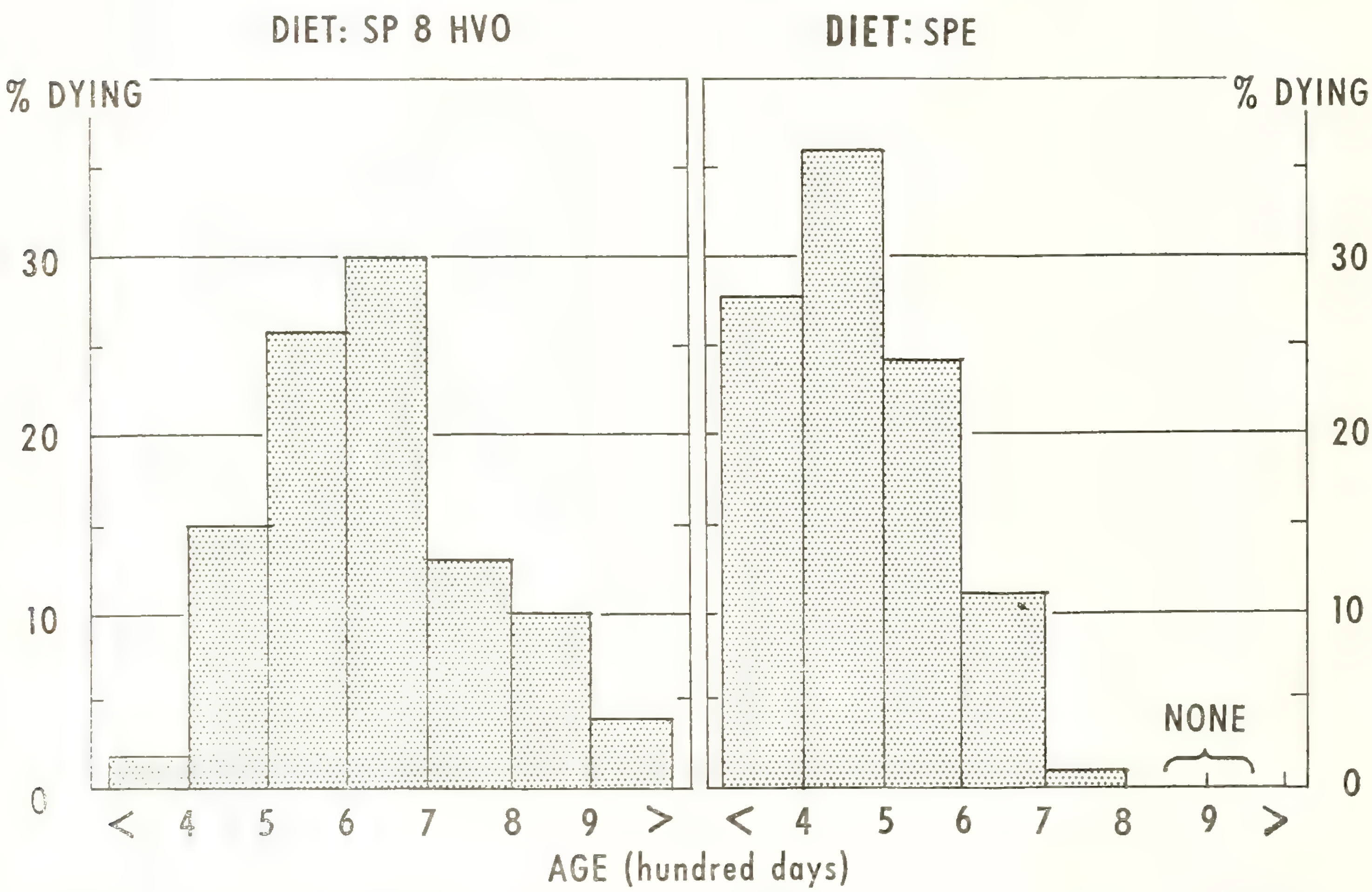

FIGURE 4.—Percentage of total number of rats dying within different age intervals on SP 8 HVO and SPE diets.

KIND AND LEVEL OF FAT.—Data available, although limited, suggest that the lifespan of rats may be influenced by the kind and/or level of fat in the semipurified diet. When the dietary fat was HVO, the average age at death was approximately the same whether the level of fat was 8 or 16 percent. Although there appeared to be no difference between the survival of rats fed diets containing 8 or 16 percent lard, the lifespan of both groups was less than that of animals fed the diets containing HVO. When butter was the dietary fat and the level was 8 percent, the average age at death was close to that observed with HVO but decreased when the diet contained 16 percent butter to approximately that observed when lard was the dietary fat.

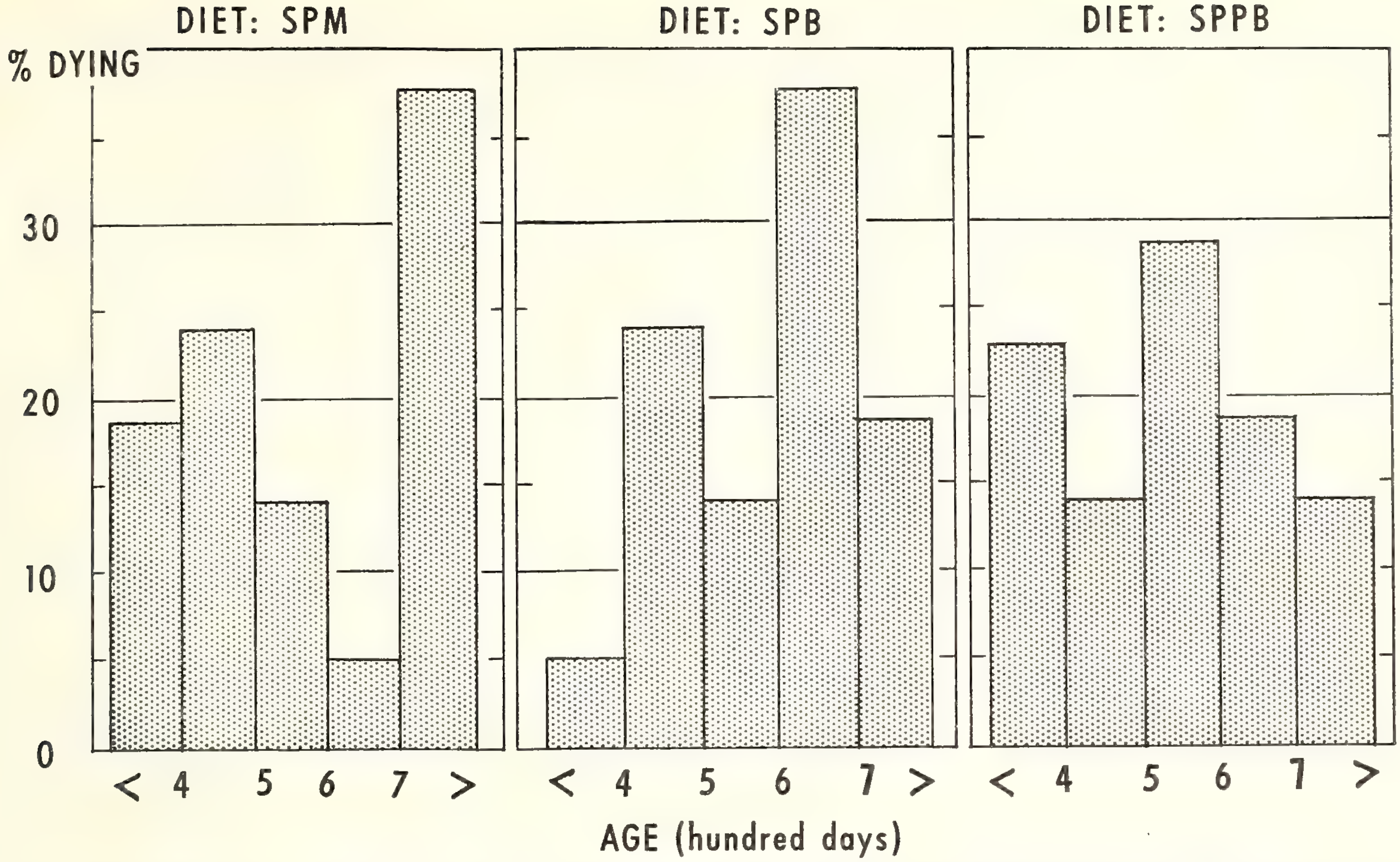

FIGURE 5.—Percentage of total number of rats dying within different age intervals on SPM, SPB, and SPPB diets.

The average length of life of rats fed SP 16 butter diet was less than that observed on SPM diet. The type and level of protein in these two diets were the same; mineral content as well as level of fat was similar. The major differences between these diets were the presence of 6 percent HVO and of 5 percent lactose from the dried skim milk in SPM diet, whereas all of the fat in SP 16 butter was from butter and all of the carbohydrate was sucrose. The presence of lactose may have been a factor contributing to the difference in the survival on the two diets, although the amount was low in comparison with the 39 percent sucrose in this diet. No antioxidants were added to these diets, and the tendency for somewhat reduced consumption of the SP 16 butter diet may be related to lower stability of the fat in this diet. The content of the low-molecular fatty acids (C_{14} and below) in the SP 16 butter was higher, about twice that of the SPM diet. Data available provide no answer as to the cause of the differences observed.

PROTEIN LEVEL.—Modification of the semipurified diet by increasing the protein or the protein and fat level to that of SPE diet resulted in no significant change in the lifespan of these rats. Respiratory infection was responsible for some of the early deaths of the small group of eight littermates fed SPa 16 HVO and SPb 8 HVO diets; no difference in survival was apparent for the larger group of littermates fed SP 8 HVO and SPa 16 HVO diets. The results with SPb 8 HVO and SPa 16 HVO were similar, suggesting again that increasing the level of HVO from 8 to 16 percent was without effect on survival.

EGG AND EGG COMPONENTS.—Rats fed a diet containing a relatively high level of egg white (SPEW) and those fed a diet consisting of 100 percent whole egg survived longer than those fed a diet containing 25 percent egg (SPE) or 30 percent egg yolk (SPEY). The lifespan of rats fed SPEW was similar to that observed for those fed the modified semipurified diet (SPa 16 HVO) with comparable levels of fat and protein, whereas the average age at death for rats fed SPEY diet was similar to that obtained for littermates fed the SPE diet containing cooked fresh egg. The survival period of rats fed 100 percent egg yolk was short. No data were obtained on longevity of rats consuming 97 percent egg yolk and 3 percent salt mixture, but other results obtained with this diet, to be considered more fully later, suggest that the lifespan of rats fed this high egg yolk diet may be increased appreciably when the diet is supplemented with a suitable salt mixture. Although some of these data seem to indicate that it is the yolk of egg that is contributing to the shortened lifespan of rats fed SPE diet, the finding that rats tolerate diets containing 100 percent egg better than a diet containing either 25 percent whole egg or 30 percent egg yolk

indicates that other dietary ingredients also are contributing to the response of rats to SPE diet.

Diet reversal.—The number of rats included in the series to investigate the influence of reversing stock and SPE diets at 250 days of age was small, and details for the individual rats are summarized in table 81 of the appendix. The harmful effects of the SPE diet appear to have been overcome or prevented for 3 of the 4 rats that were changed from SPE to stock diet at 250 days of age. The 4th rat was losing weight before the diet was changed and died shortly thereafter. This suggests that irreversible damage had already occurred before the change in dietary regimen at 250 days. The average age at death of the 3 animals that were maintaining their weight at the time of the change in diet was 705 days, in contrast to a lifespan of 473 days for rats continuing on SPE diet throughout life. Three of the 4 rats placed on SPE diet after 250 days of age lived more than 700 days, in marked contrast to 1 out of a total of 85 that reached this age when SPE diet was fed throughout life. These findings suggest that the harmful effects of SPE diet are due to a stress imposed upon the young rat, and that this diet may be well tolerated by the adult rat.

Heredity and response to SP 8 HVO or SPE diet.—Wistar rats lived longer on both SP 8 HVO and SPE diets than did BHE rats. Over 50 percent of the rats fed these diets lived more than 700 days, in marked contrast to the results with BHE rats, particularly those fed SPE diet. Although the average age of survival of Wistar rats was somewhat less on SPE than on SP 8 HVO diet, the difference was of questionable significance considering that the early death of two of the rats fed SPE diet was due to respiratory infections and seemed unrelated to diet.

The shortened lifespan that resulted from feeding SPE diet to BHE rats when their parents were raised on our regular stock diet also occurred when their parents were raised on the stock diet that had been used for the Wistar animals. Genetic differences, therefore, and not differences in dietary history of the parents appear to be responsible for the short survival of BHE rats fed SPE diet.

Stock diet.—No systematic data were collected to determine the longevity of BHE rats fed the usual stock diet, but apparently these animals are relatively long lived. Among the rats that were sacrificed to provide information on the characteristics of stock animals at different ages were 10 rats over 800 days of age. Three of these were still maintaining their weight and appeared to be in good health at 820, 916, and 976 days of age. The remaining 7 animals, from 808 to 897 days old, were losing weight at the time of sacrifice. Likewise, BHE rats fed the stock diet that had been used for raising the Wistar rats were long lived. Five rats that were continued on this diet to provide data on older animals were still maintaining their weight when sacrificed at an average age of 938 days. No information was obtained with older Wistar rats fed their usual stock diet.

Early weight gain and survival.—Although the experiments reported in this bulletin were not designed to determine the influence of food consumption and/or weight on survival, they afford considerable indirect evidence indicating that excessive food consumption and the accompanying rapid gain in body weight were important factors in determining the lifespan of these rats. In table 13 are summarized data for gross caloric intake by rats fed SP 8 HVO and SPE diets during the first 300 days of life, with the results separated into groups based on the age at which consistent weight loss began. The average intake on both diets tended to be less, particularly during the 200- to 300-day period, for rats with the longer span of healthy life, although there was considerable variation in the food intake of individual rats. All except 1 of the 9 rats fed SP 8 HVO diet with a caloric intake of 1,900 Calories or more lost weight and showed signs of ill health before they reached 600 days of age. Considering the similarity in the caloric intake of rats fed SP 8 HVO and SPE diets, however, it was apparent that differences in the lifespan on these two diets were not due to differences in the amount of food eaten by these animals.

The adverse effect of rapid weight gain in the young adult rat is still more apparent from the data shown in figure 6. Curves 1 and 2 contrast the growth curve of a group of rapidly growing rats fed SP 8 HVO diet with that of rats of relatively long lifespan; curves 3 and 4 represent similar data for rats fed SPE diet. The 14 rapidly growing rats fed SP 8 HVO diet all had attained a body weight of 550 grams by the time they reached 200 days of age; 4 exceeded 600 grams in weight. The average age at death of these rats was 553 days. Curve 2 shows the slower growth rate of 14 rats that were fed the same diet and survived 600 days or more without weight loss. Most of the rats in this latter group weighed less than 500 grams at 200 days of age, and none had reached a weight of 550 grams. The average age at death was 812 days. The 16 rapidly growing rats fed SPE diet all exceeded 600 grams in weight by 200 days, with an average age at death of 423 days. By 600 days, most of the rats fed SPE diet were dead or were losing weight, regardless of early weight gain. Curve 4, therefore, represents data for rats maintaining or gaining weight at 500 days of age. Their average lifespan of 623 days was greater than that of the rapidly growing rats fed the same diet, but did not equal that of rats growing at a comparable rate when fed SP 8 HVO diet.

Figure 7 represents similar data for a small group of rats fed SPM, SPB, and SPPB diets, and provides further evidence for the harmful effects of early rapid gain in body weight. The curves for the most rapidly growing animals fed these

Table 13.—*Maximum age of rats before weight loss in relation to caloric intake during the first 300 days on SP 8 HVO and SPE diets*

Diet and age of rats (days)	Rats	Average healthy lifespan	Intake during— 0–12 weeks Average	0–12 weeks Range	100–199 days Average	100–199 days Range	200–299 days Average	200–299 days Range
	Number	*Days*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
SP 8 HVO:								
Less than 500	15	387	6, 110	5, 260–6, 860	8, 320	7, 190– 9, 780	8, 510	7, 100– 9, 870
500 to 599	12	541	6, 000	5, 220–6, 960	8, 170	7, 280–10, 060	8, 220	7, 280– 9, 780
600 to 699	11	624	5, 870	4, 840–6, 720	7, 760	6, 160– 8, 930	7, 900	6, 530– 9, 020
700 and over	6	757	5, 590	5, 030–5, 920	7, 890	6, 670– 8, 410	7, 450	6, 720– 7, 990
SPE:								
Less than 400	20	330	6, 180	5, 540–7, 120	8, 260	6, 760–10, 180	8, 610	7, 060– 9, 290
400 to 499	11	423	6, 330	5, 690–6, 820	7, 940	6, 170– 9, 140	8, 210	6, 690–10, 180
500 and over	6	533	5, 610	5, 020–6, 340	6, 830	5, 500– 8, 100	6, 950	5, 570– 7, 730

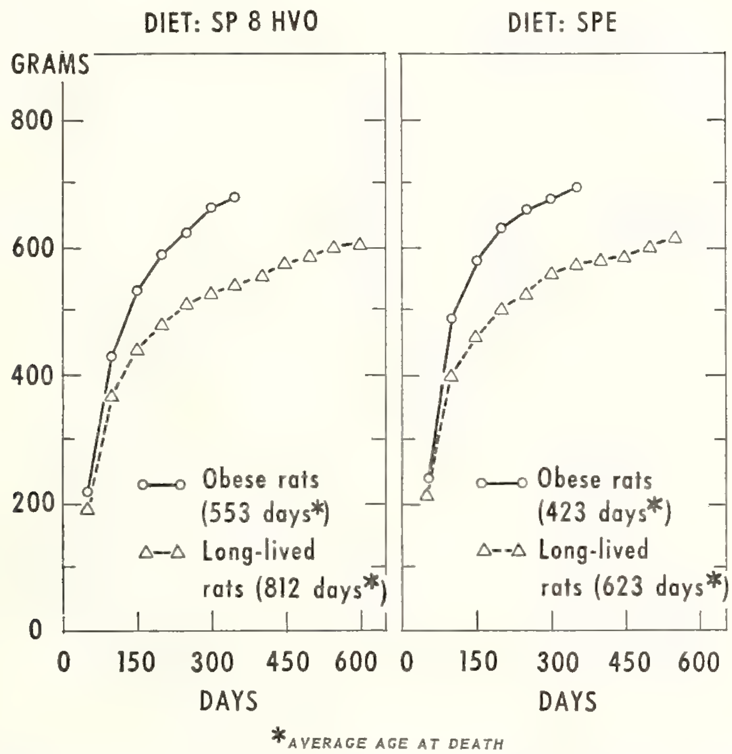

Figure 6.—Comparison of growth curves of obese rats fed SP 8 HVO and SPE diets with those of long-lived rats fed the same diets.

diets are all for rats weighing 600 grams or more by 200 days. Of the 18 rapidly growing rats fed these three diets, 8 were dead before reaching 400 days of age; only 1 survived 600 days (603 days on SPB diet). The rats that were still maintaining their weight at 600 days of age grew slowly on all three diets and survived approximately 300 days longer than did the rapidly growing rats fed the corresponding diet. The slowly growing rats tended to reach maximum weights comparable to those attained by the rapidly growing rats but at a much older age.

No data were available from this study to indicate the possible advantage of restricting food intake to prevent the excessive weight gain of the older rats fed these diets. The tendency for rats to become heavy at an early age when fed SPM and SPPB diets may be a factor in the relatively large number of early deaths on these diets. Although a rapid gain in weight was generally associated with a short lifespan, a slow rate of early gain did not necessarily result in a long, healthy life.

The results with other experimental diets show a similar trend for rapid early growth to be associated with early death, but are too few to permit their separation as has been done for the diets just discussed. The tendency to a shortened

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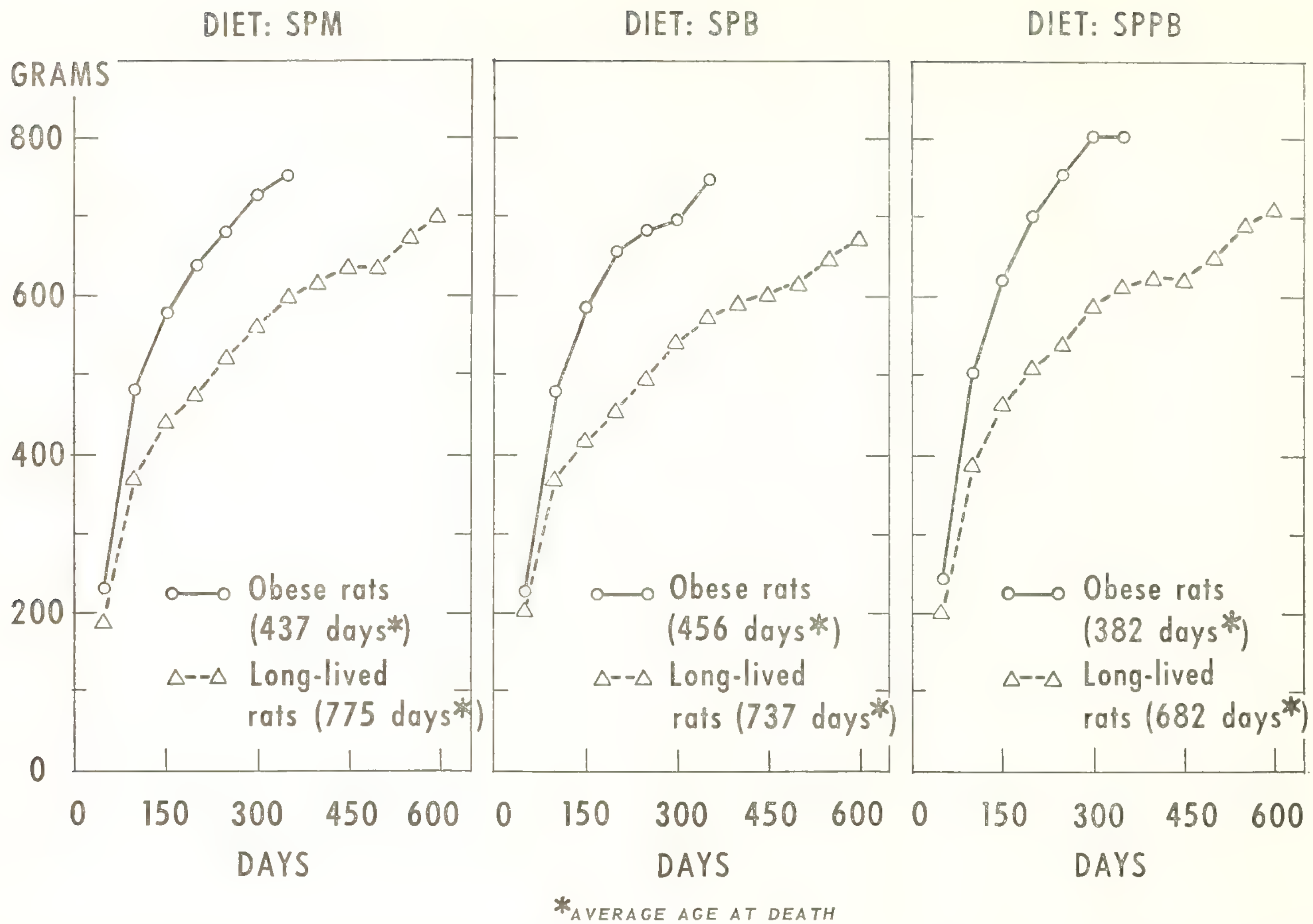

FIGURE 7.—Comparison of growth curves of obese rats fed SPM, SPB, and SPPB diets with those of long-lived rats fed the same diets.

survival period for rats fed lard (SP 8 lard and SP 16 lard) may be due in whole or in part to excessive consumption, along with the tendency to more efficient utilization of these diets.

DISCUSSION.—There have been extensive investigations on the nutritional requirements of the rat, but the majority of them have dealt with the period of early growth. Relatively few have covered the entire lifespan. Many of the early experiments concerned with longevity have dealt with diets deficient in one or more nutrients, resulting in retarded growth and in premature death. Although growth has been the basis for studying nutritional adequacy of many diets, considerable evidence has accumulated to indicate that rapid growth in early life may not insure optimum health throughout life.

McCay (*120*) was the first to demonstrate that lifespan of rats could be extended by severely restricting caloric intake while maintaining adequate levels of essential protein, minerals, and vitamins. With much less restricted intakes (33 and 46 percent) and without severe retardation of growth and sexual maturity, Berg and Simms (*21*, *22*) showed an extension of life expectancy and a delay in the onset of major diseases. Ross (*166*, *167*), investigating the effect of uniform lifelong dietary regimens on the mortality pattern of rats, demonstrated the possibility of modifying life expectancy not only by quantitative dietary restriction but also by the ratio of the protein and carbohydrate components in the diet. Riesen, Herbst, Walliker, and Elvehjem (*158*) obtained a beneficial effect on survival when the caloric intake of rats fed a synthetic diet was restricted. Carlson and Hoelzel (*41*) found that intermittent fasting tended to increase the lifespan of rats under conditions that did not result in drastic retardation of growth. Everitt and Webb (*59*) reported for male rats that the faster an animal reached its maximum weight, the sooner it deteriorated and died. Callison, Orent-Keiles, and Makower (*40*) compared the results of feeding human-type diets with rats fed a stock ration. The stock diet which produced the smallest early weight gains resulted in adult animals with the lowest maximum weight and in the best physiological condition. In contrast, the fast-growing animals which attained the highest maximum weights were inferior in physical condition, as judged by bronchiectasis and skin condition.

McCay, Maynard, Sperling, and Osgood (*121*) found that the maximum lifespan of their animals occurred when rats weighed between 350 and 450 grams. No animal with a body weight exceeding 450 grams at any time in life lived more than 810 days. In considering nutrition during the latter half of life, these authors reported that the degree of fatness of the body is the most important factor as far as lifespan is concerned.

Silberberg and Silberberg (*171*) reported the results of feeding male mice a stock diet containing 5 percent fat with or without an additional 25 percent lard. The mean lifespan of the mice consuming the fat-enriched diet throughout their life was 107 days shorter than that of the stock-fed animals. When the high-fat regimen was initiated at the age of 6 or 12 months, the lifespan was also shortened but less so than when this regimen was started at weaning. When fed for 5-month intervals, the results varied, depending on the period of life during which the fat-enriched diet was consumed. The differences observed were due to high fat and not to the caloric intake.

French, Ingram, Uram, and others (*69*) compared the results of ad libitum feeding of a diet containing 22.7 percent fat, chiefly corn oil (20 percent), with one containing 3.4 percent fat and 20 percent sucrose in place of the corn oil. The lifespan of male rats fed the high-fat diet was markedly decreased. Decreased lifespan was correlated with increased efficiency of utilization but not with caloric intake. These authors indicate that this decrease in longevity may have been due to the fat as such, or may have been the result of the improved growth rate conferred by the high-fat diet.

According to Comfort (*42*), heredity may be as important as dietary reduction in determining lifespan. Although the lifespan of laboratory animals can be increased by eliminating specific heritable diseases, inbred stock rats tend to reach a shorter lifespan than do random-bred animals, and the author suggests that information on the mechanism for vigor in hybrids may prove of significance in studies of aging. Sperling, Loosli, Barnes, and McCay (*176*) also reported data implicating inheritance as a factor in survival. Six and one-half percent of the litters of rats investigated by these authors accounted for 27 percent of the short-lived rats that died within 500 days; 18 percent of the litters accounted for 47 percent of the animals dying after 700 days. Lane and Dickie (*111*) presented data showing the shortened lifespan that results from excessive food consumption by genetically obese mice fed ad libitum and, as the result of long-term restriction of food intake of these mice, increased their lifespan by more than 300 days.

Summary

In general, growth was good on all of the experimental diets under investigation, and no evidence of any dietary deficiency was apparent. Young rats tended to grow more rapidly when the level of fat was 17 to 19 percent than when the diet contained 9 percent fat or less. With some diets, growth rate was associated with caloric intake; with others, such as those containing high levels of egg, milk, beef, or peanut butter, it appeared to be associated with efficiency of utilization.

Rats fed stock rations generally attained their maximum weight at a relatively early age and maintained a constant weight thereafter. On the semipurified diets and the various modifications of it, rats tended to continue to gain throughout their healthy lifespan. Rats exceeding 800 grams in weight were frequently obtained, especially on the diets containing milk or peanut butter.

The results reported provide further evidence of the many factors that need to be considered in evaluating the effect of diet on the lifespan of the rat. Survival of rats varied even on diets of similar fat and protein content. The tendency to excessive consumption of certain diets may explain some of the differences in survival observed. The extent of weight gain and the amount of a diet that can be tolerated by the adult rat without adverse effects appear to vary with diet. Diets containing high levels of egg or egg yolk resulted in a shortened lifespan, but the longer life of rats fed 100 percent egg indicates that other dietary ingredients were also contributing to the response to the SPE diet. The results obtained when stock and SPE diets were reversed at 250 days contribute further evidence that the age period under study may be a contributing factor in the response to diet. The results of feeding the same diets to BHE and Wistar rats emphasize the importance of recognizing inherited characteristics in evaluating dietary response.

Histology and Size of Selected Organs

Histology of kidney

When subjected to gross and to microscopic examination, the kidneys from 113 rats fed the stock diet, 99 fed SP 8 HVO diet, and 201 fed SPE diet, provided information on the influence of diet, age, fasting, and weight loss on this organ.

Rats maintaining weight on stock, SP 8 HVO, and SPE diets.—In table 14 are summarized for different age groups the results of gross and microscopic examination of the kidneys from rats that were maintaining weight on these three diets at the time of sacrifice. The results for fasted and nonfasted animals have been combined. There appeared to be a tendency toward somewhat higher ratings for the presence of hyalin casts when rats were sacrificed without fasting, but the differences were too small to warrant a separation of the results without more data to establish the significance of this trend.

TABLE 14.—*Kidney damage determined microscopically for rats maintaining weight at different ages on stock, SP 8 HVO, and SPE diets*

Diet and age of rats (days)	Rats	Gross rating	Histological rating			Kidneys with—		
			Hyalin	Cystic	Glomerular	Hyalin	Cystic	Glomerular
Stock:	*Number*	*Score*	*Score*	*Score*	*Score*	*Percent*	*Percent*	*Percent*
Less than 200	19	0	0	0	0	0	0	0
200 to 299	18	.2	.1	0	0	11	0	0
300 to 399	11	.6	.3	.1	.1	18	9	9
400 to 499	10	.6	.7	.1	.2	50	10	10
500 to 599	11	.8	1.0	.4	.5	64	27	18
600 and over	16	1.0	1.2	.4	.4	81	19	31
SP 8 HVO:								
Less than 200	5	.4	.4	0	0	20	0	0
200 to 299	12	.3	.2	0	0	17	0	0
300 to 399	8	.2	.4	0	0	38	0	0
400 to 499	7	.4	.4	0	0	29	0	0
500 to 599	9	.8	.7	.3	.3	56	11	11
SPE:								
Less than 200	5	.2	0	0	0	0	0	0
200 to 299	23	.8	1.1	.1	.04	78	0	4
300 to 399	12	1.6	1.6	.4	.1	92	33	8
400 to 499	14	1.5	1.1	.1	.2	64	14	21
500 to 599	9	2.2	1.8	.3	.3	100	33	33

In general, the kidneys of rats under 200 days of age were normal on all three diets. On *stock* or *SP 8 HVO diet* extensive degenerative changes in the kidney were rarely seen. A gradual increase occurred with age in the number of rats fed stock or SP 8 HVO diets, with kidneys showing the presence of eosinophilic albuminous material or hyalin casts. Kidneys from 81 percent of the rats fed the stock diet that were over 600 days old had a hyalin rating of 1 or more, although one of the oldest rats (916 days) had a kidney that was apparently normal. Cystic or glomerular damage was found occasionally and was seen more often in the kidneys from rats fed the stock diet than from those fed SP 8 HVO diet.

On *SPE diet* the results were in marked contrast. Of those animals that were fed this diet and were more than 200 days old, relatively few had normal kidneys. Kidneys with hyalin ratings of 2 or 3 were observed even in rats 200 to 300 days old. Cystic or glomerular damage was occasionally seen but was generally slight.

Calcium deposits were not observed in the kidneys of rats that were maintaining weight on any one of these three diets.

RATS LOSING WEIGHT ON STOCK, SP 8 HVO, AND SPE DIETS.—Both age and weight loss were found to influence the type and extent of kidney damage observed, and the data summarized in table 15 have been separated accordingly.

For rats fed the *stock diet* and with weight loss less than 100 grams, little damage was observed in the kidneys from the few animals that were less than 700 days old; extensive degenerative changes were observed in kidneys from rats over 700 days old, with 40 to 75 percent of the kidneys showing cystic and glomerular damage as well as hyalin casts. When weight loss exceeded 100 grams, 86 percent of the kidneys showed all three types of damage and the extent of the damage observed bore no relation to age. When cystic damage was large, there was a tendency for a reduction in hyalin casts. Fibrosis was generally apparent when all three types of damage were evident. Calcium was present in only one of these kidneys.

Animals fed the *semipurified* diet with weight loss less than 100 grams presented a somewhat different picture from that of animals fed the stock diet. The most extensive damage was found in kidneys from the small group of rats between 500 and 600 days of age; kidneys from animals surviving over 600 days tended to show progressively less damage with increasing age. Relatively little cystic or glomerular damage was observed. When weight loss exceeded 100 grams, only 2 of the 29 kidneys from rats 400 days of age or older were normal; no consistent relation between age and extent of damage was observed. Cystic and glomerular damage was found in 65 percent of these kidneys, a marked increase over that seen in rats losing less weight but somewhat lower than that found for comparable groups of rats fed the stock diet. Calcium deposits were found in the kidneys of 41 percent of these animals and appeared to be the heaviest between 400 and 600 days of age. Calcium was apparent only when all three types of damage were present. The data for fasted rats were too few to determine whether or not the nutritional state of the rat at the time of sacrifice was a factor in determining the presence of calcium in the kidneys of these rats.

On *SPE diet*, relatively few normal kidneys were observed, regardless of age or weight loss. For rats under 400 days of age and losing less than 100

TABLE 15.—*Kidney damage determined microscopically for rats losing weight at different ages on stock, SP 8 HVO, and SPE diets*

Weight loss, diet, and age of rats (days)	Rats	Gross damage	Histological rating				Kidneys with—			
			Hyalin	Cystic	Glomerular	Calcium	Hyalin	Cystic	Glomerular	Calcium
Rats losing less than 100 grams:										
Stock:	*Number*	*Score*	*Score*	*Score*	*Score*	*Score*	*Percent*	*Percent*	*Percent*	*Percent*
400 to 499	1	0	0	0	0	0	0	0	0	0
500 to 599	2	.2	.5	0	0	0	50	0	0	0
600 to 699	2	1.0	1.0	.5	0	0	50	50	0	0
700 to 799	4	2.2	2.0	1.0	1.8	0	75	75	75	0
800 and over	5	2.4	1.8	1.0	1.4	0	100	40	60	0
SP 8 HVO:										
Less than 300	4	0	0	0	0	0	0	0	0	0
300 to 399	2	0	0	0	0	0	0	0	0	0
400 to 499	7	.6	.9	0	0	0	57	0	0	0
500 to 599	5	2.3	2.0	1.0	.4	0	80	40	40	0
600 to 699	6	1.4	.5	.2	.2	0	33	17	17	0
700 and over	3	.7	.3	0	0	0	33	0	0	0
SPE:										
Less than 300	6	.3	.7	0	.2	0	50	0	17	0
300 to 399	21	1.8	1.8	.9	.7	.1	95	29	38	14
400 to 499	18	2.7	2.2	1.8	1.2	.3	94	72	72	17
500 to 599	9	3.8	1.9	2.6	1.3	.7	100	89	78	44
600 and over	8	3.2	2.5	2.0	1.6	.4	100	88	62	12
Rats losing more than 100 grams:										
Stock:										
300 to 399	2	4.0	2.0	2.5	3.0	1.0	100	100	100	50
400 to 499	2	3.5	2.5	2.5	2.0	0	100	100	100	0
500 to 599	3	3.3	2.7	3.0	2.7	0	100	100	100	0
600 to 699	1	4.0	4.0	2.0	3.0	0	100	100	100	0
700 to 799	4	2.0	2.0	.8	1.5	0	100	50	75	0
800 and over	2	3.5	2.5	2.0	3.0	0	100	100	100	0
SP 8 HVO:										
Less than 300	2	0	.5	0	0	0	50	0	0	0
300 to 399	--	--	--	--	--	--	--	--	--	--
400 to 499	4	3.5	2.5	2.8	1.8	1.8	100	75	75	50
500 to 599	9	2.4	2.1	1.1	1.1	1.2	100	56	67	44
600 to 699	7.	2.3	2.0	1.9	1.7	.6	86	71	71	43
700 and over	9	3.0	2.3	1.4	1.3	.3	89	67	56	22
SPE:										
Less than 300	1	4.0	3.0	1.0	1.0	2.0	100	100	100	100
300 to 399	19	3.9	2.5	3.2	2.0	2.4	100	95	95	79
400 to 499	23	3.8	2.7	3.0	2.1	1.9	100	100	100	74
500 to 599	21	3.7	2.5	3.3	1.9	1.3	100	100	95	62
600 and over	12	3.8	2.3	2.9	2.1	.9	100	100	92	42

grams in weight before sacrifice, the extent and kind of damage found was similar to that already seen (table 14) for rats that were fed this diet and were maintaining or gaining weight. In the older rats, however, there was an increase in the extent of cystic or glomerular damage. When weight loss exceeded 100 grams, no normal kidneys were seen and more than 95 percent showed glomerular and cystic damage as well as hyalin casts. Degenerative changes were extensive regardless of age, and the scores for cystic damage tended to exceed those for glomerular damage, in contrast to the results with rats fed the stock diet. Calcium was present more frequently in the kidneys of rats fed SPE diet than in those fed SP 8 HVO diet; 18 percent of the kidneys showed evidence of calcium deposition when weight loss was less than 100 grams; 62 percent, when the loss exceeded 100 grams.

Data on *SPE diet* were sufficient to permit a further evaluation of the factors influencing the occurrence of calcium deposits in the kidneys. In table 16, the results of microscopic examination for calcium are considered in relation to age, weight loss, and nutritional status of the rat at the time of sacrifice. Calcium was found occasionally in the kidneys of rats losing less than 100 grams and was frequently found in the rats losing more than 100 grams. It was observed more often in kidneys from nonfasted rats than in those from fasted rats, regardless of weight loss. When weight loss was under 100 grams, 9 percent of the kidneys from fasted rats and 32 percent from nonfasted rats

TABLE 16.—*Calcium determined microscopically in kidneys of fasted and nonfasted rats losing weight at different ages on SPE diet*

Weight loss and age of rats (days)	Fasted			Nonfasted		
	Rats	Kidney calcium	Rats with calcium in kidneys	Rats	Kidney calcium	Rats with calcium in kidneys
Rats losing less than 100 grams:	*Number*	*Score*	*Percent*	*Number*	*Score*	*Percent*
Less than 300	4	0	0	2	0	0
300 to 399	12	0	0	9	.3	33
400 to 499	13	.2	8	5	.6	40
500 to 599	8	.6	38	1	1.0	100
600 and over	6	0	0	2	1.5	50
Rats losing more than 100 grams:						
Less than 300	----	----	----	1	2.0	100
300 to 399	7	1.4	57	12	3.0	92
400 to 499	10	1.6	60	15	2.0	93
500 to 599	12	.8	50	9	2.0	78
600 and over	7	.7	43	5	1.2	40

showed evidence of calcium deposits; when weight loss was more than 100 grams, the corresponding percentages were 53 and 83. Calcium was found most frequently and in the greatest amounts in the kidneys of nonfasted rats 300 to 600 days of age that had lost more than 100 grams. Although fibrosis was not necessarily accompanied by calcium, calcium was observed only in kidneys showing extensive damage with evidence of fibrosis.

RATS FED SPM, SPB, AND SPPB DIETS.—In table 17 are summarized data from the microscopic examination of kidneys and livers from rats fed diets containing milk (SPM), beef (SPB), or peanut butter (SPPB). The results for rats that were *maintaining weight* did not differ markedly from those for rats that had lost less than 100 grams in weight before sacrifice, and the limited data for these two groups of rats have been combined. No separation by age was necessary when weight loss exceeded 100 grams.

In rats *maintaining or losing less than 100 grams* in weight, the chief evidence of degenerative changes in the kidneys was the presence of hyalin casts. On all three diets, the kidneys of rats under 300 days of age were generally normal in appearance. On SPB diet, little evidence of kidney damage was observed except in rats 500 days of age or older, whereas hyalin casts were a frequent finding in the kidneys of rats 300 to 500 days of age fed SPM or SPPB diets.

When *weight loss exceeded 100 grams,* kidney

TABLE 17.—*Kidney damage determined microscopically for rats maintaining or losing weight at different ages on SPM, SPB, and SPPB diets*

Weight loss, diet, and age of rats (days)	Rats	Average age	Histological rating of kidney			
			Hyalin	Cystic	Glomerular	Calcium
Rats maintaining weight or losing less than 100 grams:						
SPM:	*Number*	*Days*	*Score*	*Score*	*Score*	*Score*
Less than 300	16	217	0.4	0	0	0
300 to 499	12	353	.8	.3	.2	0
500 and over	9	577	.7	.2	.2	0
SPB:						
Less than 300	16	206	.1	0	.1	0
300 to 499	12	400	.3	.2	.2	0
500 and over	8	627	1.2	.5	.2	0
SPPB:						
Less than 300	19	217	.3	.2	.1	0
300 to 499	21	387	1.2	.6	.6	0
500 and over	16	597	.8	.2	.1	0
Rats losing more than 100 grams:						
SPM	14	624	1.4	1.2	.7	.6
SPB	15	556	2.3	2.1	1.3	1.1
SPPB	13	512	2.7	2.5	1.1	.5

damage was generally present, regardless of age. Kidneys from rats fed SPM diet showed the least evidence of degenerative changes. In spite of extensive damage, calcium deposits were rarely seen in the kidneys of rats fed SPPB diet although, otherwise, these kidneys were similar to those from comparable animals fed SPE diet. With regard to calcium deposition, the kidneys from rats fed SPB diet resembled more closely those from rats fed SPE diet.

Rats fed other experimental diets.—In table 18 are summarized the results of microscopic examination of the kidneys from rats fed all of the other experimental diets. The data were obtained chiefly from rats that were sick or moribund. The influence of weight loss on the extent of kidney damage was seen consistently in all of the experimental series, but for most diets the data were too limited to permit a separation of the results on this basis. The results reported, therefore, include all available data, along with the average weight loss for each group to aid in comparing the results of the various experimental regimens.

None of the *supplements* investigated were able to prevent extensive degenerative changes that occur in the kidneys of rats fed SPE diet. However, the data summarized in table 18 do show some trends suggesting that these supplements may have influenced the metabolic processes

Table 18.—*Kidney damage determined microscopically for rats fed all other diets*

Strain and diet	Rats	Average age	Average weight loss	Histological rating of kidney			
				Hyalin	Cystic	Glomerular	Calcium
BHE rats							
SPE supplemented with—	*Number*	*Days*	*Grams*	*Score*	*Score*	*Score*	*Score*
Choline, 0.5%	22	466	144	2.2	2.8	1.5	1.0
B_{12}, 0.01 mg./100 gm	7	449	136	2.0	3.0	1.9	1.3
Choline, 0.5%+B_{12}, 0.01 mg./100 gm	10	434	113	2.4	2.9	2.0	.8
B_6, 0.5 mg./100 gm	8	472	126	2.0	2.0	1.2	.4
Choline, 0.5%+B_6, 0.5 mg./100 gm	8	408	123	1.8	1.9	1.2	.6
Choline, 0.5%+B_{12}, 0.01 mg./100 gm.+B_6, 0.5 mg./100 gm	10	430	139	2.2	2.6	1.4	.3
Cholesterol, 0.46%	8	451	150	2.2	2.8	1.2	.4
Cholesterol, 1.38%	9	406	142	1.8	2.7	1.3	.1
Ascorbic acid, 0.2%	9	415	115	1.8	2.3	1.1	1.7
Ascorbic acid, 0.2%+cholesterol, 0.46%	9	434	183	2.2	3.2	1.7	1.6
SP 16 HVO	5	547	76	1.2	.2	.4	0
SP 8 lard	15	553	99	1.8	1.4	1.2	.5
SP 16 lard	8	547	120	1.8	1.1	1.1	0
SP 8 butter	6	546	76	1.8	.7	.8	.5
SP 16 butter	9	519	69	1.7	.7	.6	.4
SPa 16 HVO	17	638	173	1.6	1.5	1.2	.5
SPb 8 HVO	9	632	118	1.7	1.0	.9	.3
SPEW	6	530	155	2.5	1.8	1.0	1.3
SPEY	7	474	178	2.3	3.1	1.6	1.9
SPW 8 HVO	5	550	0	.6	0	0	0
E100	20	535	135	1.5	2.3	1.4	.6
Y100	16	403	77	.6	.9	.2	1.6
Littermates fed—							
SPE	5	428	66	2.4	1.4	1.0	.8
Y100	5	425	35	.8	.6	.4	2.0
Y97+salt mixture, 3.0%	5	430	5	.4	0	0	0
Littermates fed—							
Stock	3	590	195	2.7	2.7	3.0	0
SPE	2	396	133	2.0	3.5	1.5	1.5
Stock changed to SPE at 250 days	4	686	109	2.2	1.8	1.0	.8
SPE changed to stock at 250 days	3	577	101	2.0	1.7	1.7	.3
Wistar rats							
Littermates fed—							
SP 8 HVO	9	729	106	.4	.3	.2	0
SPE	9	664	78	.6	.6	.4	0
BHE parents fed Wistar stock diet							
Young fed—							
Stock	5	886	0	.8	.2	.4	.8
SP 8 HVO	9	703	75	1.7	1.0	.8	.2
SPE	7	500	85	2.1	2.3	1.4	.6

involved in the utilization of these diets. Cystic damage appeared to be somewhat reduced when vitamin B_6 was added to the diet, with or without choline. Calcium ratings were particularly high for rats fed the diets containing supplements of ascorbic acid with or without added cholesterol.

When the *level or kind of fat, or both,* were varied, the extent of the kidney damage observed was more like that found with the semipurified diet than with SPE diet. Damaged kidneys were seen frequently but no marked differences were observed in these moribund rats that could be ascribed to the kind or level of fat. Extensive degenerative changes, apparent from gross examination of five kidneys that were not suitable for microscopic examination, indicate that the lower rating for the kidneys from rats fed SP 16 HVO was without significance. When the *level of protein* was increased, the results were similar to the results with SP 8 HVO diet when differences in weight loss before sacrifice were considered.

Microscopically, the kidneys and livers of rats fed the diet containing 30 percent egg yolk (SPEY) appeared similar to those from comparable rats fed SPE diet. The damage observed in the kidneys from rats fed the diet containing egg white (SPEW) was less extensive than that observed with SPE diet and resembled that seen in moribund rats fed the semipurified diet. Less extensive kidney damage occurred when the diet contained 100 percent whole egg than when the diet contained 25 percent egg (SPE), paralleling the longer survival of rats fed E100 diet.

The microscopic appearance of the kidneys from rats fed 100 percent egg yolk differed significantly from that observed with the other diets containing whole egg or egg fractions. Cystic or glomerular damage was small; calcium deposits were heavy, even in rats showing relatively small weight loss, and were not necessarily associated with extensive cystic damage or fibrosis. Most of the rats fed 100 percent egg yolk were sacrificed before weight loss exceeded 100 grams, but there was no evidence of a marked increase in cystic or glomerular damage with increasing weight loss. The results from microscopic examination of tissues from rats fed diets consisting solely of whole egg or containing high levels of egg yolk or egg white contrast with those for rats fed 25 percent egg (SPE), and provide further evidence that ingredients in the SPE diet other than egg must be contributing to the acceleration of the degenerative changes that occur on this diet.

Further evidence of this was obtained from a group of 15 rats, 5 fed SPE diet, 5 fed 100 percent egg yolk, and 5 fed 97 percent egg yolk supplemented with 3 percent salt mixture. The rats were scheduled for sacrifice at 450 days of age. When a rat on any one of the three diets became moribund and was sacrificed before 450 days, the corresponding littermates fed the other diets were sacrificed at the same time. Two of the rats fed SPE diet became moribund at 396 and 398 days of age, and 1 rat fed 100 percent egg yolk was losing weight before reaching 450 days of age. The 5 rats fed egg yolk supplemented with the salt mixture appeared healthy at the time they were sacrificed. The histological findings for the kidneys from these rats suggest that the shortened lifespan of rats fed a diet containing 100 percent egg yolk was due, in part at least, to a mineral imbalance and was not accompanied by marked degenerative changes such as were seen in the kidneys of rats fed SPE diet. The kidneys of rats fed the egg yolk diet supplemented with 3 percent of a salt mixture containing the mineral elements considered essential were generally normal in appearance and showed no evidence of calcium deposition.

In table 18 are also included the results of microscopic examination of tissues from rats that were changed after 250 days from stock to SPE diet or the reverse. The kidneys from rats that were maintained throughout life on stock or SPE diet were typical of those already discussed for these diets, with the high glomerular damage in the kidneys from rats fed the stock diet and high cystic damage and heavy calcium deposits in those from rats fed SPE diet. When the diet was reversed, kidney damage was less extensive with both diets, and these animals lived longer than did the other littermates that were continued on either stock or SPE diet.

Influence of heredity.—Wistar rats were much less susceptible to kidney damage than were the BHE strain. Of the nine kidneys examined from rats fed SP 8 HVO diet, only one from a rat 848 days old was badly damaged; on SPE diet, two of the nine kidneys showed marked degenerative changes, one from a rat 397 days old and one from a rat 846 days old. No calcium deposits were seen on either of these diets, and the marked difference in the response of BHE rats to SP 8 HVO and SPE diets was not apparent with Wistar rats. The differences in the response of these two strains of rats did not appear to be due to the dietary history of the parent rats. A similar difference in the response of BHE rats to SP 8 HVO or SPE diets was observed whether the parents were raised on the usual stock diet or on the diet that was used to raise the Wistar stock rats.

Discussion.—A tendency for chronic nephritis to occur in the older rat has been observed by many investigators. The extent of the kidney damage found was generally less than that reported in this publication for BHE rats. Results reported elsewhere have dealt chiefly with the occurrence of nephritis in older rats without regard to diet. Saxton and Kimball (*168*) found chronic nephrosis in 44 percent of the kidneys from rats dying naturally, and considered that this condition occurred spontaneously in the albino rat. Degenerative changes in the kidneys were rarely seen in young rats, and the incidence of kidney damage increased with age up to 800 days. There was a

tendency to fewer damaged kidneys in animals surviving more than 800 days of age. When growth was retarded throughout life, nephrosis was almost nonexistent.

Berg and Harmison (*20*) and Simms and Berg (*172*), investigating a stock colony from which respiratory infections had practically been eliminated, reported that chronic nephrosis, the commonest pathological condition observed, was present in 80 percent of the animals by 700 days. Kennedy (*102*) also observed severe renal damage in rats surviving 2 years or longer with little evidence of renal abnormalities before they reached an age of 21 months. Renal damage appeared earlier, between 12 and 15 months, in obese rats. Overfeeding or unilateral nephrectomy resulted in the premature appearance of a type of kidney lesion common to senile rats. Andrew and Pruett (*10*) compared normal kidneys of rats in age groups 300 days and younger with kidneys from rats over 800 days of age. The greatest differences observed were in the tubules rather than in the glomeruli. Blatherwick and Medlar (*30*) presented evidence that functional impairment of the kidney may exist for some time before histologic changes indicative of nephritis become apparent. Gover (*77*) reported differences in the type of renal lesions observed in three strains of mice.

Dietary studies in relation to kidney damage have dealt chiefly with the influence of protein level and have yielded somewhat controversial results. Bischoff (*29*) reviewed the results of the early investigations in this field. Casein has been studied most frequently in experiments dealing with high levels of dietary protein. There seems to be considerable evidence that rats under many conditions can consume relatively high levels of casein for the greater part of the normal lifespan without suffering renal lesions characteristic of nephritis. Saxton and Kimball (*168*), however, observed chronic nephrosis more often in animals receiving casein than in those receiving liver. Nephrosis was frequently greater in rats that received diets high in protein than in those on low protein diets. Although chronic nephrosis was more common in animals on high levels of protein there appeared to be no correlation of lifespan with the level of dietary protein.

A few reports have also dealt with the possible role of magnesium in the production of renal damage. A diet containing cholesterol and cholic acid and producing atherosclerosis in the rat increases markedly the magnesium requirement of the animal (*187*). Vitale, Hellerstein, Hegsted, and others (*186*), in reviewing the present knowledge of the interrelationship between dietary magnesium and calcium in atherosclerosis and renal lesions, reported that additional magnesium decreases or eliminates calcium deposition in the kidney, regardless of other variables.

Summary.—Gross examinations of the tissues at the time of necropsy indicated that the kidney was the organ most frequently abnormal. Kidney damage was observed in rats showing no obvious signs of ill health as well as in sick animals, but the extent of the damage was considerably greater in the moribund rats.

Microscopic examination revealed three types of kidney damage. The most prevalent finding was the presence of eosinophilic albuminous material or hyalin casts in dilated tubules. As evidence of degenerative changes increased, glomerular damage became apparent, and in the large, excessively damaged kidneys, extreme dilation of the tubules resembling cystic degeneration was observed. Calcium deposits were found chiefly in the kidneys of moribund rats and were rarely seen except in kidneys showing extensive damage.

The BHE strain of rats seemed to be particularly susceptible to kidney damage regardless of the diet, and the results suggest that several factors may accelerate an inherent weakness in this strain of rats. Some diets obviously hastened the onset of lesions and appeared to influence the type and extent of the degenerative changes observed. The levels of protein in the stock and experimental diets were moderate and were similar except for those diets consisting of 100 percent whole egg or egg yolk. Level of protein, therefore, does not explain the excessive kidney damage observed with rats fed SPE or SPPB diets. Level of dietary fat also provides no explanation for the results observed.

The tendency for kidney damage to be less when the diet consisted solely of whole egg or egg yolk supplemented with a mineral mixture than when it contained 25 percent whole egg indicates that the undesirable effect of this latter diet was due to the interaction of the various dietary ingredients rather than to egg alone. The results with the mineral-supplemented egg-yolk diet suggest the possibility of a mineral imbalance.

The enlarged and damaged kidneys observed in some extremely heavy animals, particularly those fed the diet containing peanut butter, may be due to an acceleration of degenerative changes in the kidneys, associated with the stress of obesity.

The results provide no answer as to why calcium deposits were present with certain diets but were absent in equally damaged kidneys from rats fed other experimental diets. The diets were designed to supply the animal with adequate amounts of vitamins and minerals, but the possibility that certain dietary stresses may increase the need for some of these nutrients has not been excluded.

Wistar rats proved much less susceptible to kidney damage than did BHE rats and responded very differently to the diet containing 25 percent egg. These results provide further evidence of the importance of considering inherent characteristics of the strain of animal under investigation when interpreting the results of nutritional investigations, and of the need for further research to discover the biochemical mechanisms involved.

Histology of liver

Stock, SP 8 HVO, and SPE diets.—Microscopic examination of the livers from 115 rats fed the *stock diet* showed very little pathology. Periductal infiltration and edema were noted occasionally in the older animals. Only one of the livers examined showed the presence of fat vacuoles.

The results from examination of 98 livers from rats fed the *SP 8 HVO diet* in general were similar to those for rats fed the stock diet except for the occasional appearance of small amounts of fat. Of these livers, 14 showed microscopic evidence of fat, and 10 of the 14 were from moribund rats that were more than 600 days old. In no case were the livers excessively fatty.

In contrast to the results with rats fed stock or SP 8 HVO diet, microscopic examination indicated that most of the livers from rats fed *SPE diet* were fatty. In table 19 are summarized the results from the histological ratings for fat of 202 livers from rats fed SPE diet. Relatively high ratings for fat were obtained for livers from rats, regardless of age, that were maintaining their weight at the time of sacrifice. In rats over 500 days of age, the score for large vacuoles as well as for small vacuoles was high. Only 7 of the 58 rats over 200 days of age had livers showing no evidence of fat. Fat was also seen in two of the five livers from young nonfasted rats approximately 150 days old. The presence of glycogen in livers from nonfasted rats as well as protein that may be lost during fasting was responsible for the somewhat lower rating for fat in the livers of nonfasted rats 200 to 400 days old. There was a tendency for the scores for liver fat to be lower for moribund rats, particularly for animals losing more than 100 grams in weight. No evidence of fat was observed in livers from 27 of the 78 rats in this latter group.

SPM, SPB, and SPPB diets.—The results from microscopic examination of the livers for fat in rats fed SPM, SPB, and SPPB diets are summarized in table 20. Excessively fatty livers were rarely seen with any of these three diets. On *SPM diet,* fat was observed most frequently in the older rats; the larger amounts were seen in the livers of moribund rats over 700 days old and losing more than 100 grams. On *SPB diet,* relatively little fat was apparent regardless of the age or general health of the rat. On *SPPB diet,* small amounts of fat were generally observed in rats over 400 days of age that were maintaining weight but, in contrast to the results with SPM diet, fat was not detected in the livers of the older moribund rats that were losing over 100 grams.

All other diets.—In table 21 are summarized the results of microscopic examination of the livers from rats, chiefly sick or moribund, fed all of the other experimental diets. None of the supplements added to SPE diet were able to prevent the tendency to fatty livers with this diet. Although interpretation of data on liver fats in moribund rats is complicated by variable weight losses, there appears to be evidence for exceedingly fatty livers in rats fed SPE diet to which cholesterol was

Table 19.—*Liver fat determined microscopically for fasted and nonfasted rats maintaining or losing weight at different ages on SPE diet*

Weight status and age of rats (days)	Fasted					Nonfasted				
	Rats	Histological rating		Livers with—		Rats	Histological rating		Livers with—	
		Small vacuoles	Large vacuoles	Small vacuoles	Large vacuoles		Small vacuoles	Large vacuoles	Small vacuoles	Large vacuoles
Rats maintaining weight:	*Number*	*Score*	*Score*	*Percent*	*Percent*	*Number*	*Score*	*Score*	*Percent*	*Percent*
Less than 200	-------	-------	-------	-------	-------	5	0.6	0.2	40	20
200 to 299	7	2.3	0.3	86	29	16	1.8	.4	88	44
300 to 399	8	3.0	.9	100	87	4	1.2	.8	100	75
400 to 499	14	2.1	.9	79	57	-------	-------	-------	-------	-------
500 and over	9	2.4	2.1	89	100	-------	-------	-------	-------	-------
Rats losing less than 100 grams:										
200 to 299	4	1.0	0	50	0	2	1.5	0	50	0
300 to 399	12	1.4	.8	58	58	8	2.4	1.0	88	75
400 to 499	13	1.4	1.5	62	92	5	2.0	1.0	80	80
500 and over	14	1.5	1.6	71	93	3	1.3	1.0	67	100
Rats losing more than 100 grams:										
200 to 299	-------	-------	-------	-------	-------	1	0	0	0	0
300 to 399	7	1.3	.9	71	57	12	.3	.7	17	67
400 to 499	10	.9	1.4	50	90	15	.9	.8	40	60
500 and over	19	.9	1.1	47	63	14	.6	1.0	36	57

TABLE 20.—*Liver fat determined microscopically for rats fed SPM, SPB, and SPPB diets*

Weight status, diet, and age of rats (days)	Rats	Average age	Average weight loss	Histological rating of liver fat	
				Small vacuoles	Large vacuoles
Rats maintaining weight:					
SPM:	*Number*	*Days*	*Grams*	*Score*	*Score*
Less than 400	16	240	0	0. 2	0. 1
400 to 599	5	497	0	. 6	. 2
SPB:					
Less than 400	16	241	0	0	. 1
400 to 599	6	489	0	0	. 5
SPPB:					
Less than 400	15	232	0	0	0
400 to 599	9	516	0	. 8	. 9
Rats losing less than 100 grams:					
SPM	17	449	47	. 1	. 1
SPB	15	446	51	0	. 1
SPPB	29	429	52	. 3	. 3
Rats losing more than 100 grams:					
SPM	14	624	175	. 5	. 8
SPB	17	572	175	. 1	. 1
SPPB	13	502	177	0	0

added. There was no evidence that the presence of fat droplets in the liver was related to the kind or level of fat in the diet. There was a tendency to fatty livers with all of the diets containing whole egg or egg yolk, although the livers of rats consuming 100 percent egg appeared to contain less fat than those of rats fed the diet containing 25 percent egg (SPE). Although the addition of salt mixture to diet Y100 seemed to prevent the occurrence of extensive kidney damage, the liver fat of the animals fed this diet, with or without the mineral supplement, seemed to be similar. Relatively little fat was apparent microscopically in the livers of rats changed from stock to SPE diet at 250 days; when the reverse procedure was used (SPE to stock at 250 days), liver fat resembled that seen in animals continued on SPE diet throughout life.

Although there was a tendency for more fat in the livers of *Wistar rats* fed SPE diet than for this same strain of rat fed SP 8 HVO diet, the amount of fat appeared to be less than that generally found in BHE rats fed SPE diet.

Quantitative data concerning fat in the liver as influenced by diet and age are provided by chemical analyses and are reported in a later section of this publication in which the problem of liver fat is considered in greater detail.

Kidney and liver weight

KIDNEY WEIGHT OF ANIMALS MAINTAINING WEIGHT ON STOCK, SP 8 HVO, AND SPE DIETS.—In table 22 are summarized for different age groups the weight of the kidneys from rats that were consuming stock, SP 8 HVO, or SPE diets and that were maintaining weight at the time of sacrifice. Included are data comparing the weights of kidneys from rats that were fasted for 17 hours and bled by cardiac puncture, with those from rats that were neither fasted nor bled at the time of sacrifice.

On *stock diet*, kidney size of fasted rats apparently varied little with age. The average kidney weight of 1.56 grams represented 0.36 percent of the body weight. The largest kidney obtained weighed 2.10 grams, and only 3 of the 38 rats had kidneys exceeding 2.0 grams in weight. The relation between kidney and body weight was relatively constant for all age groups. In contrast, kidneys from nonfasted rats tended to increase in size with age and to represent an increase in relation to body weight from 0.36 percent in young rats to 0.47 percent in rats over 600 days old. The kidneys from these rats tended to be larger than those from fasted rats of comparable age, exceeding 2.0 grams in 9 of the 11 rats that were over 600 days old.

On the *semipurified diet*, the kidneys from 200- to 499-day-old fasted rats were similar in weight to those from rats fed the stock diet but were somewhat larger in the older rats. Kidneys exceeding 2.0 grams in weight were obtained from 35 percent of the rats that were between 500 and 600 days old. In relation to body weight, the kidneys were significantly smaller ($P < 0.01$) than those from rats fed the stock diet. Data for nonfasted rats fed SP 8 HVO diet were limited to animals less than 400 days old, but kidneys tended to be large even in these young rats. Kidney weights for rats 300 to 399 days old were similar to those for rats under 200 days old, in spite of the increase in body weight of approximately 100 grams.

On *SPE diet*, the kidneys of fasted rats tended to be larger than those from comparable stock or

TABLE 21.—*Liver fat determined microscopically for rats fed other experimental diets*

Strain and diet	Rats	Average age	Average weight loss	Histological rating of liver fat	
				Small vacuoles	Large vacuoles
BHE rats					
SPE supplemented with—	*Number*	*Days*	*Grams*	*Score*	*Score*
Choline, 0.5%	22	466	144	1. 1	0. 5
B_{12}, 0.01 mg./100 gm	7	449	136	. 4	. 6
Choline, 0.5% + B_{12}, 0.01 mg./100 gm	10	434	113	. 4	. 6
B_6, 0.5 mg./100 gm	8	472	126	1. 1	. 9
Choline, 0.5% + B_6, 0.5 mg./100 gm	8	408	123	. 8	. 6
Choline, 0.5% + B_{12}, 0.01 mg./100 gm. + B_6, 0.5 mg./100 gm	10	430	139	1. 2	. 9
Cholesterol, 0.46%	8	451	150	1. 3	2. 7
Cholesterol, 1.38%	9	406	142	1. 6	2. 1
Ascorbic acid, 0.2%	9	415	115	. 6	. 8
Ascorbic acid, 0.2% + cholesterol, 0.46%	9	434	183	. 4	1. 0
SP 16 HVO	5	547	76	0	0
SP 8 lard	15	553	99	. 1	. 3
SP 16 lard	8	547	120	. 1	0
SP 8 butter	6	546	76	. 5	. 5
SP 16 butter	9	519	69	0	. 1
SPa 16 HVO	17	698	173	. 2	1. 5
SPb 8 HVO	9	632	118	. 2	. 6
SPEW	6	530	155	. 2	0. 5
SPEY	7	474	178	. 9	1. 3
SPW 8 HVO	5	550	0	. 2	. 4
E100	20	535	135	. 2	. 8
Y100	16	403	77	. 8	. 5
Littermates fed—					
SPE	5	428	66	1. 2	. 4
Y100	5	425	35	1. 0	. 8
Y97 + salt mixture, 3.0%	5	430	5	1. 2	. 8
Littermates fed—					
Stock	3	590	195	0	0
SPE	3	473	104	0	. 7
Stock changed to SPE at 250 days	4	686	109	1. 0	1. 0
SPE changed to stock at 250 days	3	577	101	0	. 3
Wistar rats					
Littermates fed—					
SP 8 HVO	9	729	106	. 1	0
SPE	9	664	78	. 4	. 7
BHE parents fed Wistar stock diet					
Young fed—					
Stock	5	886	0	0	0
SP 8 HVO	9	703	75	. 2	. 2
SPE	7	560	85	1. 4	1. 4

SP 8 HVO rats. More than 50 percent of the kidneys from rats over 300 days of age exceeded 2.0 grams in weight. In nonfasted animals fed this diet, kidneys tended to increase in size with age. Two of the rats 300 to 399 days old had kidneys weighing 8.9 grams.

LIVER WEIGHT OF ANIMALS MAINTAINING WEIGHT ON STOCK, SP 8 HVO, AND SPE DIETS.—In table 23 are summarized comparable data for the livers from rats fed stock, SP 8 HVO, or SPE diets. On the *stock diet*, there was no indication that age influenced appreciably the size of the livers from fasted rats. The average liver weight for these 37 rats was 11.1 grams, representing 2.6 percent of body weight.

The livers from nonfasted rats tended to increase in size with age, representing 3.4 percent of body weight in rats less than 200 days old and 3.9 percent in rats 600 days of age and older. The livers from these nonfasted rats were heavier than those from the fasted rats, and the extent of the differences in the weights of this organ between these two groups of rats depended upon age. Livers from nonfasted rats less than 300 days old were approximately 30 percent heavier than those from fasted rats of comparable age. In rats over 500 days old, the difference amounted to approximately 60 percent. The range of values for liver weights of the nonfasted rats was wide, even within age groups, with an extremely wide range of values for rats exceeding 300 days of age. The results for the distribution seen in table 23, however, show that the increase in liver size of nonfasted rats with age was due to the increase in

TABLE 22.—*Kidney weights in fasted and nonfasted rats maintaining weight at different ages on stock, SP 8 HVO, and SPE diets*

Condition, diet, and age (days)	Rats	Average age	Average weight at death[1]	Kidney weight		Kidney to body weight	Rats with kidneys weighing—			
				Average	Range		Less than 1.50 grams	1.50 to 1.99 grams	2.00 to 2.99 grams	3.00 and over
Fasted rats										
Stock:	*Number*	*Days*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Less than 200	4	160	381	1.34	1.12–1.50	0.35	75	25	0	0
200 to 299	15	248	447	1.58	1.13–1.92	.35	27	73	0	0
300 to 399	6	382	428	1.58	1.17–2.02	.36	33	50	17	0
400 to 499	5	436	436	1.54	1.32–1.82	.34	60	40	0	0
500 and over	8	727	410	1.66	1.21–2.10	.37	25	62	12	0
SP 8 HVO:										
200 to 299	6	252	502	1.41	1.24–1.74	.28	83	17	0	0
300 to 399	8	353	541	1.57	1.23–2.39	.29	50	38	12	0
400 to 499	7	457	600	1.63	1.19–2.59	.26	57	29	14	0
500 to 599	14	546	680	2.17	1.43–5.24	.32	7	57	29	7
SPE:										
200 to 299	7	252	552	1.69	1.46–1.81	.31	14	86	0	0
300 to 399	8	348	588	2.44	1.60–3.99	.41	0	38	50	12
400 to 499	14	455	616	2.29	1.47–4.50	.37	14	36	29	21
500 to 599	11	530	624	2.34	1.76–3.82	.36	0	36	54	9
Nonfasted rats										
Stock:										
Less than 200	15	148	390	1.41	.98–1.86	.36	73	27	0	0
200 to 299	11	258	466	1.67	1.38–2.01	.36	27	54	18	0
300 to 399	5	376	482	2.01	1.34–2.96	.41	20	40	40	0
400 to 499	7	465	486	1.87	1.07–2.70	.38	14	57	29	0
500 to 599	11	538	490	2.15	1.41–3.61	.43	9	54	27	9
600 and over	11	679	457	2.23	1.45–2.93	.47	9	9	82	0
SP 8 HVO:										
Less than 200	5	154	521	2.08	1.79–2.54	.40	0	60	40	0
200 to 299	6	248	532	2.02	1.43–3.32	.37	17	50	17	17
300 to 399	23	332	636	1.88	1.41–2.71	.30	13	61	26	0
SPE:										
Less than 200	5	154	520	1.92	1.76–2.17	.37	0	60	40	0
200 to 299	18	277	613	2.22	1.49–4.19	.37	6	22	67	6
300 to 399	29	330	614	3.01	1.69–8.88	.49	0	38	38	24

[1] Weight before 17-hour fast.

the percentage of rats with large livers and not to an occasional excessively heavy liver.

The increasing difference with age between both the kidney and the liver weight of fasted and nonfasted animals suggests that a slowing of the metabolic processes has occurred in the older rats, resulting in a temporary enlargement of these organs, which can still return to normal after a 17-hour fast.

In fasted rats fed *SP 8 HVO diet*, livers showed a consistent tendency to increase in size with age, in contrast to the relatively constant values observed for the weights of livers from comparable stock rats. The livers represented 2.1 percent of body weight, with no apparent influence of age on this relationship. The increase in liver size with age, therefore, seems to be due chiefly to the tendency for adult rats fed SP 8 HVO diet to continue gaining in body weight with age. Although the livers from these animals weighed more, on the average, than those from stock rats, they were significantly smaller in relation to body weight ($P<0.01$). There was no evidence that age was a factor within the limited range for which data were available.

Livers from nonfasted rats averaged 20.3 grams in weight, 8 grams heavier than those from fasted rats of corresponding age. In nonfasted rats fed this diet, large livers were observed even in rats less than 200 days old. In contrast to the stock rats, the livers from these nonfasted SP 8 HVO rats tended to represent a smaller percentage of the body weight with increasing age, 4.5 percent for rats under 200 days, and 3.1 percent for those between 300 and 400 days of age.

On *SPE diet*, the livers from fasted rats were larger than those from rats fed stock or SP 8 HVO diets, averaging 21.0 grams in weight and 3.4 percent of body weight. The range of values was wide, from 12.8 grams for the smallest liver to 31.8

TABLE 23.—*Liver weights in fasted and nonfasted rats maintaining weight at different ages on stock, SP 8 HVO, and SPE diets*

Condition, diet, and age (days)	Rats	Average age	Average weight at death [1]	Liver weight		Liver to body weight	Rats with livers weighing—			
				Average	Range		Less than 10.0 grams	10.0 to 14.9 grams	15.0 to 19.9 grams	20.0 grams and over
Fasted rats										
Stock:	*Number*	*Days*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Less than 200	4	160	381	9.6	8.3–10.2	2.5	50	50	0	0
200 to 299	15	248	447	11.5	8.3–14.3	2.6	20	80	0	0
300 to 399	6	382	428	11.7	8.6–16.1	2.6	33	50	17	0
400 to 499	5	436	436	11.2	9.1–12.2	2.5	20	80	0	0
500 and over	7	723	405	10.7	8.7–13.6	2.4	43	57	0	0
SP 8 HVO:										
200 to 299	6	252	502	11.0	9.1–13.9	2.2	33	67	0	0
300 to 399	8	353	541	12.0	9.6–14.1	2.2	12	88	0	0
400 to 499	7	457	600	13.4	10.1–18.2	2.2	0	71	29	0
500 to 599	14	546	680	14.3	10.8–17.9	2.1	0	71	29	0
SPE:										
200 to 299	7	252	552	18.2	12–8–24.3	3.3	0	14	71	14
300 to 399	8	348	588	23.6	18.2–30.1	3.9	0	0	12	88
400 to 499	14	455	616	21.7	14.3–31.8	3.5	0	7	29	64
500 to 599	11	530	624	20.0	13.5–26.6	3.1	0	9	46	46
Nonfasted rats										
Stock:										
Less than 200	15	148	390	13.3	9.4–15.8	3.4	87	13	0	0
200 to 299	11	258	466	15.1	12.0–17.8	3.2	46	54	0	0
300 to 399	5	376	482	16.8	14.2–21.7	3.4	20	60	20	0
400 to 499	7	465	486	17.7	14.4–25.9	3.6	29	57	14	0
500 to 599	11	538	490	18.6	13.0–26.9	3.7	18	46	36	0
600 and over	11	679	457	18.7	11.7–21.7	3.9	9	54	36	0
SP 8 HVO:										
Less than 200	5	154	521	23.7	21.2–30.7	4.5	0	0	80	20
200 to 299	6	248	532	18.7	14.3–27.2	3.5	17	50	33	0
300 to 399	23	332	636	20.0	14.6–26.3	3.1	9	39	52	0
SPE:										
Less than 200	5	154	520	22.7	19.2–27.1	4.3	0	20	80	0
200 to 299	18	277	613	28.4	21.8–36.4	4.6	0	0	72	28
300 to 399	25	331	617	29.9	20.0–48.1	4.8	0	0	60	40

[1] Weight before 17-hour fast.

grams for the largest. No consistent trend with age was noted except for the larger percentage of rats less than 300 days old with livers weighing less than 20 grams.

The livers from nonfasted rats fed SPE diet averaged 28.6 grams in weight and represented 4.7 percent of body weight. For rats less than 200 days old, livers were large in comparison to those from rats fed the stock diet but similar in size to those from comparable SP 8 HVO rats. The largest livers obtained with stock, SP 8 HVO, or SPE rats were from nonfasted SPE rats 200 to 399 days old.

CORRELATION COEFFICIENTS.—The data in tables 22 and 23 show that the factors affecting kidney weight generally exert a parallel influence on liver weight. In figure 8 are seen the lines obtained from the regression equation expressing the relationship between kidney and liver weights of fasted and nonfasted rats fed stock diet as well as the data for individual rats. Food intake immediately before sacrifice was not controlled for the nonfasted rats and undoubtedly accounts, in part at least, for the greater variation in the relationship observed for these rats.

For any one diet, liver and kidney weights tended to parallel body weight, and variations in body weight within age groups seemed to account in part for the wide range of values observed in the weight of these organs. Consideration of the individual data, however, indicated that the close parallelism between liver and kidney weights was not due entirely to the relation of the weight of these organs to body weight. In table 24 are summarized, for rats fed stock and SP 8 HVO diets, data relating liver to kidney weight and these organs to body weight. Correlation coefficients of 0.84 to 0.88 were obtained relating liver and kidney weights of fasted and of nonfasted rats fed these two diets. Correlation coefficients relating liver

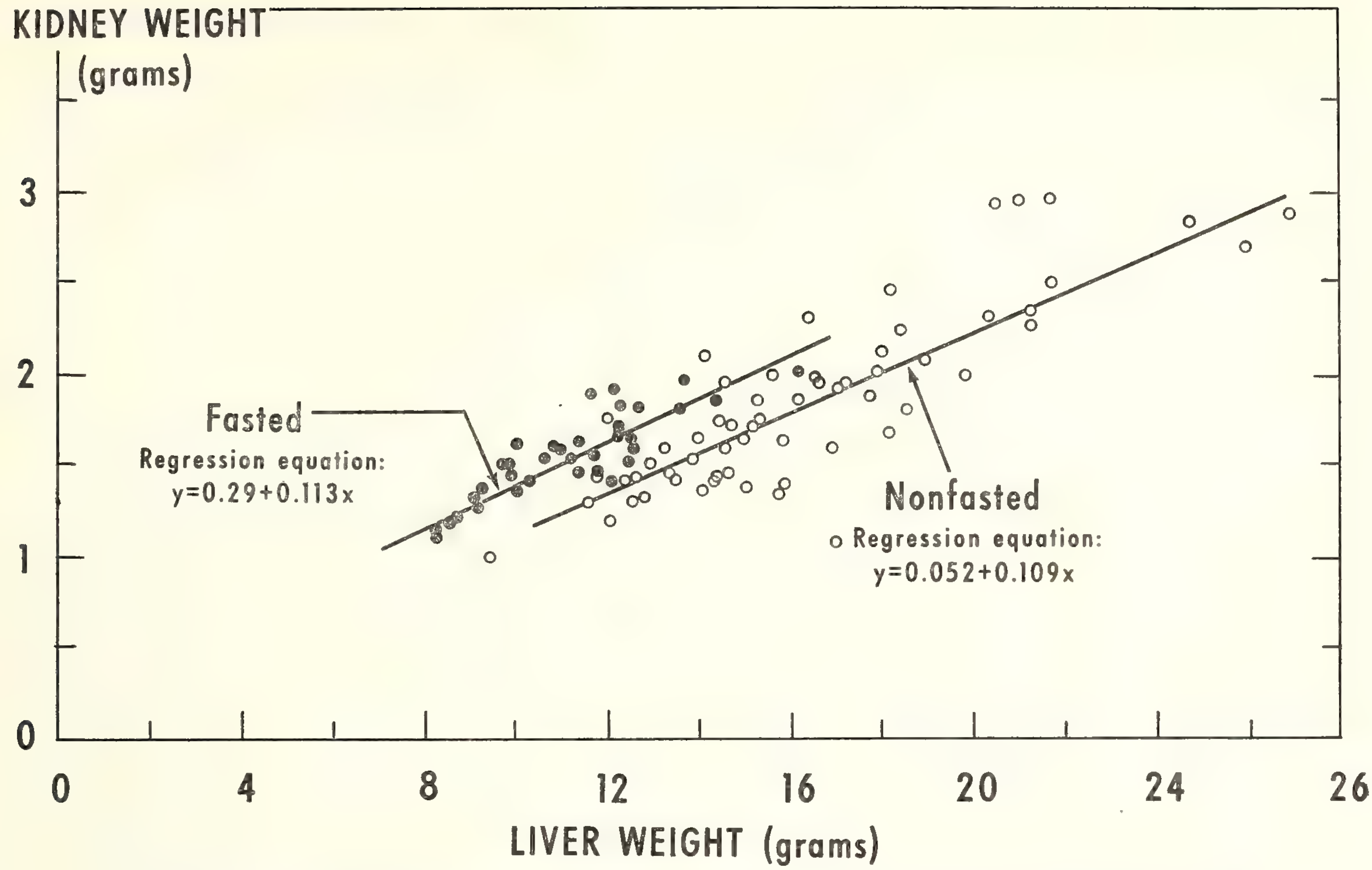

FIGURE 8.—Liver weight in relation to kidney weight in fasted and nonfasted rats fed stock diet.

or kidney weight to body weight tended to be lower than those for liver and kidney, although for fasted rats the differences observed were generally small. The lowest correlation coefficients were those relating liver or kidney to body weight in nonfasted rats fed the semipurified diet.

There was little evidence for a quantitative relationship between liver and kidney weights or between these organs and body weight in rats fed SPE diet. Even in fasted rats, ratios for liver to kidney weight varied from 3.4 to 15.5.

KIDNEY WEIGHT OF ANIMALS LOSING WEIGHT ON STOCK, SP 8 HVO, AND SPE DIETS.—In table 25 are summarized data on the kidney weights of fasted and nonfasted rats that were fed stock, SP 8 HVO, or SPE diets and were losing weight when sacrificed. The results for rats losing less than 100 grams have been separated from those for rats losing over 100 grams in weight.

When weight loss was less than 100 grams, the kidneys from fasted rats fed *stock* or *SP 8 HVO diets* were similar in size to those from rats of comparable age that were maintaining weight on these diets. On *SPE diet*, however, large kidneys were observed even in young rats, and in animals 400 to 599 days old 68 percent of the kidneys weighed more than 4 grams. When weight loss exceeded 100 grams, the kidneys from fasted rats tended to be larger with all three diets than did those from comparable animals with little or no weight loss. The largest kidneys were from rats fed SPE diet.

The tendency of the nonfasted rats to have larger kidneys than the fasted animals was apparent among those that were losing weight, as well as among those maintaining weight, although the differences were small for older SPE rats when exceedingly large kidneys were found. In nonfasted rats losing more than 100 grams, kidneys were large at all ages regardless of diet; kidneys from SPE rats tended to be larger than those from animals fed stock or SP 8 HVO diets.

LIVER WEIGHT OF ANIMALS LOSING WEIGHT ON STOCK, SP 8 HVO, AND SPE DIETS.—In table 26 are summarized data for the liver weights of rats that were losing weight on stock, SP 8 HVO, or SPE diets. In general, the results for rats losing less than 100 grams were similar to those for comparable rats that were maintaining weight except for the tendency to smaller livers in young nonfasted rats fed SP 8 HVO diet. When weight loss exceeded 100 grams, the difference between the liver weights of fasted and nonfasted rats became small, reflecting the reduced food intake of these animals. The livers from SPE rats were consistently larger than those from rats fed stock or SP 8 HVO diets. The marked increase in kidney weight that has been seen to occur with increasing loss in body weight was not accompanied by a parallel increase in liver weight.

Table 24.—*Correlation of liver, kidney, and body weights in fasted and nonfasted rats fed stock and SP 8 HVO diets*

Condition and diet	Rats	Liver to kidney weight		Liver to body weight		Kidney to body weight	
		Ratio	Correlation coefficient	Percent	Correlation coefficient	Percent	Correlation coefficient
Fasted rats:	*Number*						
Stock	37	7. 2	0. 88	2. 5	0. 71	0. 35	0. 72
SP 8 HVO	34	7. 6	. 85	2. 1	. 80	. 28	. 60
Nonfasted rats:							
Stock	59	8. 9	. 85	3. 5	. 74	. 40	. 60
SP 8 HVO	33	10. 7	. 84	3. 4	. 49	. 31	. 45

Table 25.—*Kidney weights in fasted and nonfasted rats losing weight at different ages on stock, SP 8 HVO, and SPE diets*

Weight loss, condition, diet, and age (days)	Rats	Average age	Kidney weight		Rats with kidneys weighing—				
			Average	Range	Less than 1.50 grams	1.50 to 1.99 grams	2.00 to 2.99 grams	3.00 to 3.99 grams	More than 4.00 grams
Rats losing less than 100 grams:									
Fasted:									
Stock:	*Number*	*Days*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Less than 500	----	----	----	----	----	----	----	----	----
500 and over	7	718	1. 89	1. 62– 2. 54	0	71	29	0	0
SP 8 HVO:									
Less than 500	7	295	1. 30	. 81– 1. 71	71	29	0	0	0
500 and over	10	644	1. 84	1. 46– 2. 59	10	60	30	0	0
SPE:									
Less than 400	19	296	2. 18	. 85– 7. 79	37	32	21	0	11
400 to 599	22	489	5. 73	1. 85–11. 2	0	9	18	5	68
600 and over	6	643	3. 38	1. 79– 6. 22	0	17	50	17	17
Nonfasted:									
Stock:									
Less than 500	1	456	1. 48	1. 48	100	0	0	0	0
500 and over	8	787	2. 72	2. 17– 3. 70	0	0	62	38	0
SP 8 HVO:									
Less than 500	10	375	1. 79	1. 33– 2. 21	20	50	30	0	0
500 and over	8	645	3. 28	1. 81– 6. 32	0	25	25	25	25
SPE:									
Less than 400	11	316	4. 36	1. 51–13. 3	0	18	36	18	27
400 to 599	7	498	5. 30	1. 78–12. 4	0	14	14	29	43
600 and over	2	608	8. 60	5. 78, 11. 4	0	0	0	0	100
Rats losing more than 100 grams:									
Fasted:									
Stock:									
Less than 500	----	----	----	----	----	----	----	----	----
500 and over	4	682	2. 74	2. 09– 3. 95	0	0	75	25	0
SP 8 HVO:									
Less than 500	3	419	4. 90	1. 53– 8. 67	0	33	0	0	67
500 and over	4	654	2. 92	1. 86– 4. 49	0	50	0	25	25
SPE:									
Less than 400	7	376	7. 74	3. 56–13. 2	0	0	0	14	86
400 to 599	22	504	6. 93	3. 37–11. 6	0	0	0	5	95
600 and over	7	644	4. 98	2. 79– 7. 07	0	0	14	0	86
Nonfasted:									
Stock:									
Less than 500	4	411	4. 50	2. 69– 8. 05	0	0	50	0	50
500 and over	7	695	3. 35	1. 65– 5. 98	0	29	0	43	29
SP 8 HVO:									
Less than 500	2	426	3. 35	2. 17, 4. 53	0	0	50	0	50
500 and over	21	685	3. 81	1. 27– 7. 80	5	19	19	19	38
SPE:									
Less than 400	13	339	8. 13	4. 80–12. 6	0	0	0	0	100
400 to 599	27	486	7. 14	3. 06–12. 2	0	0	0	15	85
600 and over	5	643	6. 32	3. 50– 8. 11	0	0	0	20	80

Table 26.—*Liver weights in fasted and nonfasted rats losing weight at different ages on stock, SP 8 HVO, and SPE diets*

Weight loss, condition, diet, and age (days)	Rats	Average age	Liver weight		Rats with livers weighing—				
			Average	Range	Less than 10.0 grams	10.0 to 14.9 grams	15.0 to 19.9 grams	20.0 to 29.9 grams	More than 30.0 grams
Rats losing less than 100 grams:									
Fasted rats:									
Stock:	*Number*	*Days*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Less than 500	---	---	---	---	---	---	---	---	---
500 and over	6	712	12. 5	10. 7–15. 5	0	83	17	0	0
SP 8 HVO:									
Less than 500	7	295	9. 4	6. 5–11. 7	57	43	0	0	0
500 and over	9	645	12. 4	9. 1–15. 4	11	78	11	0	0
SPE:									
Less than 400	19	296	16. 1	7. 6–26. 0	16	37	21	26	0
400 to 599	22	489	20. 7	15. 9–27. 0	0	0	50	50	0
600 and over	6	643	19. 6	16. 5–25. 6	0	0	67	33	0
Nonfasted rats:									
Stock:									
Less than 500	1	456	10. 3	10. 3	0	100	0	0	0
500 and over	8	787	19. 3	14. 6–24. 1	0	12	50	38	0
SP 8 HVO:									
Less than 500	10	375	14. 3	10. 0–23. 0	0	70	20	10	0
500 and over	5	619	20. 1	14. 0–24. 7	0	20	20	60	0
SPE:									
Less than 400	11	315	25. 4	15. 0–38. 7	0	0	18	55	27
400 to 599	6	489	26. 7	15. 1–38. 9	0	0	33	33	33
600 and over	2	608	43. 1	33. 6, 52. 6	0	0	0	0	100
Rats losing more than 100 grams:									
Fasted rats:									
Stock:									
Less than 500	---	---	---	---	---	---	---	---	---
500 and over	4	682	15. 4	11. 9–18. 2	0	50	50	0	0
SP 8 HVO:									
Less than 500	3	419	14. 7	13. 1–18. 0	0	67	33	0	0
500 and over	4	654	13. 8	13. 8–14. 0	0	100	0	0	0
SPE:									
Less than 400	7	376	21. 2	14. 0–30. 2	0	14	43	29	14
400 to 599	22	504	19. 7	14. 7–27. 5	0	5	55	41	0
600 and over	7	644	16. 5	14. 3–19. 8	0	28	72	0	0
Nonfasted rats:									
Stock:									
Less than 500	4	411	19. 8	14. 0–23. 1	0	25	0	75	0
500 and over	7	695	16. 6	14. 6–19. 4	0	29	71	0	0
SP 8 HVO:									
Less than 500	2	426	14. 7	14. 2, 15. 2	0	50	50	0	0
500 and over	20	695	16. 0	10. 8–22. 4	0	35	50	15	0
SPE:									
Less than 400	13	339	20. 5	16. 4–24. 1	0	0	38	62	0
400 to 599	25	482	20. 5	14. 3–40. 1	0	8	48	40	4
600 and over	5	643	20. 2	16. 1–26. 9	0	0	60	40	0

Kidney weight and damage.—Table 27 shows the relation of kidney size to the histological findings. Because of the influence of fasting on the size of this organ, the results of fasted and nonfasted rats are reported separately. For both fasted and nonfasted rats, a gradual increase in kidney damage with increasing kidney weight was observed with all three diets. Kidneys weighing less than 1.8 grams were generally normal in appearance. The only evidence of degenerative changes in kidneys weighing 1.8 to 2.0 grams was the presence of a few hyalin casts. When kidneys weighed between 2 and 3 grams, more extensive damage was apparent, with glomerular damage occurring in many of the kidneys from stock rats. Kidneys weighing more than 3 grams generally showed evidence microscopically of all three types of damage regardless of diet, but the ratings for glomerular damage were consistently greater for rats fed the stock diet.

Liver weight, liver fat, and kidney damage.—The weights of livers from rats fed the

TABLE 27.—*Kidney weight as related to kind and extent of damage in fasted and nonfasted rats fed stock, SP 8 HVO, and SPE diets*

Condition, diet, and range of kidney weight (grams)	Rats	Average kidney weight	Histological rating		
			Hyalin	Cystic	Glomerular
Fasted rats					
Stock:	*Number*	*Grams*	*Score*	*Score*	*Score*
Less than 1.80	25	1. 44	0. 1	0	0
1.80 to 1.99	2	1. 89	1. 0	0	0
2.00 to 2.99	7	2. 31	1. 9	. 9	1. 4
3.00 to 4.99	1	2. 75	4. 0	2. 0	3. 0
SP 8 HVO:					
Less than 1.80	34	1. 44	. 2	0	0
1.80 to 1.99	7	1. 89	. 7	0	0
2.00 to 2.99	6	2. 38	1. 2	. 2	. 2
3.00 to 4.99	3	4. 12	2. 3	2. 0	2. 3
5.00 and over	1	8. 67	3. 0	4. 0	2. 0
SPE:					
Less than 1.80	23	1. 55	. 6	0	0
1.80 to 1.99	10	1. 87	1. 1	0	. 1
2.00 to 2.99	25	2. 53	2. 0	. 5	. 2
3.00 to 4.99	16	4. 22	2. 5	1. 9	1. 5
5.00 and over	43	7. 44	2. 4	3. 4	2. 0
Nonfasted rats					
Stock:					
Less than 1.80	34	1. 47	. 1	0	0
1.80 to 1.99	10	1. 90	. 7	. 1	0
2.00 to 2.99	23	2. 38	1. 4	. 7	. 7
3.00 to 4.99	9	3. 57	3. 0	2. 0	2. 9
5.00 and over	2	7. 02	2. 0	4. 0	3. 0
SP 8 HVO:					
Less than 1.80	12	1. 62	. 4	0	0
1.80 to 1.99	7	1. 90	. 6	0	0
2.00 to 2.99	12	2. 34	1. 2	. 2	. 2
3.00 to 4.99	11	4. 18	2. 6	2. 2	1. 8
5.00 and over	5	6. 82	3. 6	3. 0	2. 4
SPE:					
Less than 1.80	7	1. 70	. 6	0	0
1.80 to 1.99	5	1. 87	1. 0	0	0
2.00 to 2.99	21	2. 28	1. 2	0	. 1
3.00 to 4.99	13	3. 77	2. 5	1. 2	1. 4
5.00 and over	40	8. 52	2. 4	3. 5	2. 2

stock diet also paralleled closely the extent of the damage observed in the kidneys. In table 28 are summarized data from nonfasted stock rats that were maintaining their body weight. Little evidence of kidney damage was observed in these rats when livers weighed less than 16 grams; extensive damage was found when livers exceeded 20 grams in weight. There were too few data to establish a comparable relationship in the fasted rats, and in moribund rats this relationship was complicated by variable weight loss.

In nonfasted rats fed *SP 8 HVO diet*, larger livers were not necessarily accompanied by damaged kidneys. No evidence of kidney damage was apparent in the group of five rats 150 days of age, although three of the livers from these rats weighed more than 20 grams.

On *SPE diet* no consistent relation was found between kidney damage and liver size or between size of liver or fat in the liver as seen microscopically. A liver weighing 13.5 grams showed evidence of fat in amounts comparable to that seen in livers weighing 25 grams. Fat was apparent at an early age before kidney damage occurred, and was seen in all but one of the livers from rats with normal kidneys. As kidney

TABLE 28.—*Liver weight as related to kind and extent of kidney damage in nonfasted rats maintaining weight on stock diet*

Liver weight range (grams)	Rat	Average liver weight	Histological rating		
			Hyalin	Cystic	Glomerular
	Number	*Grams*	*Score*	*Score*	*Score*
Less than 14.0	15	12. 5	0	0	0
14.0 to 15.9	18	14. 9	. 1	0	0
16.0 to 17.9	11	17. 0	. 9	0	0
18.0 to 19.9	6	18. 6	1. 0	. 3	. 3
20.0 and over	10	22. 8	2. 1	1. 2	1. 6

damage increased, liver fat tended to decrease and often was too small to be apparent microscopically without special stains for fat.

Rats maintaining weight on SPM, SPB, and SPPB diets.—In table 29 are summarized data for the weights of the livers and kidneys from fasted and nonfasted rats that were maintaining their weight on diets containing milk, beef, or peanut butter. For comparison, data are also included for rats fed SP 8 HVO and SPE diets from the same experimental series. The younger fasted or nonfasted rats were littermates except for an occasional death before the age scheduled for sacrifice.

The influence of fasting on the size of the kidney as well as the liver was again apparent in the results for the young rats. In general, the results for SPM, SPB, and SPPB diets were similar to those obtained with the semipurified diet except for the tendency to large livers in animals over 400 days old fed SPPB diet. For all diets except the SPE diet, an increase in liver weight with age was accompanied by a parallel increase in body weight, with the liver remaining a relatively constant percentage of the body weight. The tendency for certain litters to be particularly prone to enlarged, damaged kidneys was apparent in the group of rats 400 to 600 days of age. One litter accounted for the largest kidney recorded in table 29 for each of the diets except SPE. The littermate fed SPE diet was moribund at the time of sacrifice and had a kidney weighing 8.7 grams. The kidney weighing 5.57 grams from the littermate fed SPB diet was responsible for the high average ratio of kidney to body weight noted for this diet.

Rats losing weight on SPM, SPB, and SPPB diets.—Differences among these diets became apparent in rats that were losing weight, as is seen in table 30. Some extremely large kidneys were found in young rats fed SPPB diet, particularly in the nonfasted animals. The largest kidney observed, weighing 15.8 grams, was from a 341-day-old nonfasted rat fed this diet. Although large kidneys were a frequent finding in SPPB rats, the kidneys of a small group of older nonfasted rats that had lost less than 100 grams were normal in size. This group of animals included the oldest surviving rats on this diet. Enlarged kidneys were a frequent finding when weight loss exceeded 100 grams, regardless of diet, although there were relatively few large kidneys among the older SPM rats. The relation of kidney size to the extent and type of damage observed was similar to that previously discussed for rats fed SP 8 HVO or SPE diets; hyalin casts were generally present in kidneys weighing 2 grams or more and cystic and glomerular damage in those exceeding 3 grams in weight.

The livers from SPPB-fed rats tended to be large but were generally smaller than those from SPE-fed rats. In contrast to the results with SPE diet, the large livers from rats fed SPPB diet generally showed little microscopic evidence of fat.

Rats fed SPE diet with added nutrients.—In table 31 are summarized data for the kidney and liver weights of rats fed the 10 supplemented SPE diets. Enlarged kidneys and livers such as

Table 29.—*Kidney and liver weights of fasted and nonfasted rats maintaining weight at different ages on diets with protein-fat-containing foods*

Condition, age of rats (days), and diet	Rats	Average age	Kidney weight		Liver weight		Average body weight	Kidney to body weight	Liver to body weight	Liver to kidney weight
			Average	Range	Average	Range				
Fasted rats										
200 to 399 days:	*Number*	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Ratio*
SP 8 HVO	5	313	1. 37	1. 23–1. 60	10. 7	9. 1–13. 1	523	0. 27	2. 1	7. 8
SPE	5	313	2. 09	1. 60–2. 91	21. 7	17. 0–30. 1	578	. 36	3. 7	10. 6
SPM	6	304	1. 38	1. 06–1. 85	11. 9	7. 3–16. 2	580	. 24	2. 0	8. 6
SPB	6	304	1. 52	1. 16–2. 23	11. 9	8. 7–15. 5	540	. 28	2. 2	7. 8
SPPB	6	289	1. 43	1. 12–1. 91	11. 8	10. 4–14. 3	495	. 29	2. 4	8. 6
400 to 499 days:										
SP 8 HVO	10	523	2. 01	1. 43–2. 59	14. 6	11. 9–18. 2	716	. 28	2. 0	7. 4
SPE	6	496	2. 09	1. 55–3. 82	18. 9	14. 3–24. 4	648	. 32	2. 9	9. 9
SPM	7	503	1. 90	1. 43–3. 20	13. 8	10. 4–19. 6	659	. 29	2. 1	7. 3
SPB	8	502	2. 16	1. 33–5. 57	13. 8	9. 4–18. 8	642	. 34	2. 1	6. 6
SPPB	8	509	2. 06	1. 46–3. 68	16. 9	13. 0–22. 5	741	. 28	2. 3	8. 5
Nonfasted rats										
150 to 250 days:										
SP 8 HVO	10	201	2. 08	1. 43–3. 32	21. 6	14. 3–30. 7	530	. 39	4. 1	10. 5
SPE	10	201	2. 04	1. 76–2. 22	23. 9	19. 2–27. 1	540	. 37	4. 4	11. 8
SPM	10	202	1. 98	1. 51–2. 34	21. 2	16. 5–27. 5	581	. 34	3. 7	10. 7
SPB	10	202	1. 90	1. 55–2. 24	18. 6	16. 5–21. 9	540	. 35	3. 4	9. 8
SPPB	10	202	1. 96	1. 59–2. 85	20. 0	15. 0–27. 7	569	. 34	3. 5	10. 3

TABLE 30.—*Kidney and liver weights of fasted and nonfasted rats losing weight at different ages on SPM, SPB, and SPPB diets*

Condition, weight loss, age (days), and diet	Rats	Average age	Kidney weight		Liver weight [1]	
			Average	Range	Average	Range
Fasted						
Losing less than 100 grams:						
200 to 499 days old:	*Number*	*Days*	*Grams*	*Grams*	*Grams*	*Grams*
SPM	5	350	1. 63	0. 99– 2. 60	13. 4 (4)	7. 7 –17. 2
SPB	3	268	1. 22	. 85– 1. 47	9. 9	7. 3 –11. 9
SPPB	17	330	2. 26	. 56– 7. 41	15. 2	5. 0 –21. 5
500 days and older:						
SPM	5	528	2. 50	1. 93– 4. 35	16. 2	14. 7 –18. 1
SPB	4	651	2. 52	1. 63– 4. 53	15. 6	12. 7 –20. 2
SPPB	5	586	3. 25	1. 38– 7. 09	18. 2	11. 0 –24. 7
Losing more than 100 grams:						
200 to 499 days old:						
SPM	1	362	1. 10	1. 10	9. 63	9. 6
SPB	1	461	1. 51	1. 51	12. 5	12. 5
SPPB	----------	----------	----------	----------	----------	----------
500 days and older:						
SPM	3	796	2. 97	1. 63– 5. 53	13. 0	10. 1 –15. 6
SPB	4	630	2. 82	1. 50– 3. 48	12. 2	11. 0 –14. 8
SPPB	4	531	3. 80	2. 12– 5. 78	19. 4 (3)	12. 5 –28. 8
Nonfasted						
Losing less than 100 grams:						
200 to 499 days old:						
SPM	7	354	2. 67	1. 30– 6. 28	18. 2	14. 7 –22. 3
SPB	7	312	2. 05	. 96– 3. 70	16. 5 (6)	8. 7 –27. 7
SPPB	6	373	5. 50	1. 52–15. 8	22. 5	9. 8 –33. 1
500 days and older:						
SPM	2	728	2. 23	1. 83, 2. 63	19. 4	18. 0, 20. 8
SPB	3	696	2. 53	1. 64– 3. 36	22. 8	15. 2 –31. 3
SPPB	6	643	1. 98	1. 77– 2. 28	19. 4	15. 2 –25. 8
Losing more than 100 grams:						
200 to 499 days old:						
SPM	5	433	4. 46	1. 48– 8. 68	16. 5	12. 0 –21. 8
SPB	5	435	4. 93	3. 01– 6. 40	16. 0	13. 9 –18. 1
SPPB	5	397	6. 06	3. 84– 7. 99	22. 2	11. 8 –29. 2
500 days and older:						
SPM	7	706	2. 94	1. 58– 4. 41	15. 1 (5)	11. 4 –18. 3
SPB	9	609	5. 08	2. 00– 8. 03	16. 3	13. 4 –21. 4
SPPB	4	606	5. 20	3. 89– 6. 25	18. 4	14. 2 –21. 9

[1] Numbers in parentheses indicate when number of livers do not correspond to number of kidneys.

have generally been observed in rats fed SPE diet were also obtained with all of the supplemented diets. The kidneys from rats fed the diets supplemented with vitamin B_6 tended to be smaller, on the average, than those from rats fed the unsupplemented diet, paralleling the histological findings. The largest average kidney weights were obtained when the diets were supplemented with vitamin B_{12} or with cholesterol. Although these supplements were without effect on the lifespan of these animals, there is some indication that they may have influenced the metabolic processes involved in the utilization of these diets. Considering the wide range of values obtained in moribund rats, however, data uncomplicated by excessive weight loss are needed to establish the significance of the trends observed.

RATS FED DIETS CONTAINING 8 OR 16 PERCENT HVO, LARD, OR BUTTER.—The kidney and liver weights for rats fed diets in which the kind and/or level of fat was varied are summarized in table 32. Data from animals that were maintaining weight are limited to a group of five nonfasted rats fed SP 8 HVO diet and the corresponding results with the littermates fed SP 8 lard. No significant differences in the liver or kidney weights of these young rats were obtained. The results for sick or moribund rats have been separated on the basis of weight loss before sacrifice. When weight loss was less than 100 grams, the kidneys from rats fed diets containing hydrogenated vegetable oil at either the 8- or 16-percent level tended to be smaller than those from rats fed diets containing 8 or 16 percent lard or butter. When weight loss exceeded 100 grams, the results for fasted and for nonfasted rats have been combined because of the small differences usually observed in these two groups of animals. The kidneys from this latter

TABLE 31.—*Kidney and liver weights of rats losing weight on SPE diet containing added purified nutrients*

SPE supplement	Rats	Average age	Average weight loss	Kidney weight		Rats	Average age	Average weight loss	Liver weight	
				Average	Range				Average	Range
	Number	*Days*	*Grams*	*Grams*	*Grams*	*Number*	*Days*	*Grams*	*Grams*	*Grams*
None	18	452	120	5. 66	1. 78–11. 6	16	458	122	21. 2	14. 3–32. 9
Choline, 0.5%	20	452	142	6. 16	1. 90–10. 9	18	457	144	20. 9	14. 5–30. 8
B_{12}, 0.01 mg./100 gm.	10	447	139	6. 73	2. 29–15. 1	8	462	131	21. 0	15. 8–27. 7
Choline, 0.5%+B_{12}, 0.01 mg./100 gm.	10	434	114	7. 18	2. 40–12. 7	10	434	114	24. 9	16. 0–43. 0
B_6, 0.5 mg./100 gm.	9	464	120	4. 53	2. 12–12. 2	8	472	126	20. 3	16. 0–26. 9
Choline, 0.5%+B_6, 0.5 mg./100 gm.	9	412	128	4. 74	1. 62– 7. 8	9	412	128	19. 6	13. 7–26. 4
Choline, 0.5%+B_{12}, 0.01 mg./100 gm.+B_6, 0.5 mg./100 gm.	10	430	139	5. 76	2. 11–10. 3	10	430	139	19. 9	16. 6–25. 4
Cholesterol, 0.46%	9	458	153	7. 47	2. 56–11. 0	9	458	153	22. 8	17. 6–33. 5
Cholesterol, 1.38%	10	408	142	6. 83	2. 07–12. 8	10	408	142	22. 2	15. 7–33. 6
Ascorbic acid, 0.2%	9	421	133	5. 51	2. 40–10. 4	8	436	130	19. 2	12. 8–29. 5
Ascorbic acid, 0.2%+cholesterol, 0.46%	10	422	170	5. 40	2. 37– 7. 7	9	434	183	20. 8	14. 4–37. 0

TABLE 32.—*Kidney and liver weights of fasted and nonfasted rats maintaining or losing weight on diets containing different kinds and levels of fat*

Condition, weight status, and diet	Rats	Average age	Average weight loss	Kidney weight		Liver weight	
				Average	Range	Average	Range
Nonfasted rats							
Maintaining weight:	*Number*	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
SP 8 HVO	5	184	0	1. 95	1. 76–2. 54	22. 3	16. 3–30. 7
SP 8 lard	5	186	0	1. 83	1. 64–2. 48	18. 9	17. 4–21. 7
Fasted rats							
Losing less than 100 grams:							
SP 8 HVO	5	703	53	1. 61	1. 46–1. 69	12. 6	11. 8–15. 0
SP 16 HVO	4	700	54	1. 77	1. 32–3. 45	14. 2	12. 3–15. 5
SP 8 lard	6	632	48	2. 78	1. 70–4. 91	16. 3	13. 8–18. 8
SP 16 lard	2	695	94	2. 84	2. 15, 3. 52	15. 4	15. 1, 15. 6
SP 8 butter	4	605	32	2. 15	1. 59–3. 45	14. 0	13. 1–15. 4
SP 16 butter	5	554	56	2. 65	1. 67–5. 95	14. 6	13. 0–16. 4
Fasted and nonfasted rats							
Losing more than 100 grams:							
SP 8 HVO	7	587	151	4. 50	1. 86–8. 67	15. 3	11. 3–19. 0
SP 16 HVO	2	636	152	4. 94	2. 64, 7. 24	15. 2	14. 4, 16. 1
SP 8 lard	6	493	163	5. 12	1. 50–7. 93	15. 6	11. 7–18. 5
SP 16 lard	5	555	138	4. 04	3. 47–4. 99	15. 7	11. 7–18. 0
SP 8 butter	4	591	152	4. 02	1. 80–6. 18	[1] 14. 4 (3)	13. 9–15. 0
SP 16 butter	1	404	147	5. 10	5. 10	13. 0	13. 0

[1] Number in parentheses indicates number of animals when differing from that listed in column 1.

group of animals were generally large regardless of the kind or level of dietary fat.

RATS FED DIETS WITH PROTEIN AND FAT OF SP 8 HVO ADJUSTED TO LEVEL IN SPE DIETS.—In table 33 are summarized data for kidney and liver size when rats were fed modifications of the semipurified diet in which the level of fat was increased to 16 percent and protein to 30 percent, or the level of protein alone was increased. Most of these rats had lost considerable weight before death. The results obtained were similar to those observed under comparable conditions with rats fed SP 8 HVO diet. The smaller kidneys of the rats fed SPB diet were accompanied by smaller weight losses. From these data, there appeared no evidence that an increase in the level of fat or protein resulted in kidneys and livers comparable in size to those from rats fed SPE diet, at least as far as could be ascertained with moribund rats.

RATS FED DIETS CONTAINING WHOLE EGG, EGG WHITE, OR EGG YOLK.—In table 34 are summarized data for rats that were fed diets containing whole egg, egg white, or egg yolk. Included are data for 10 rats fed SPE diet when the egg in this diet was dried, cooked fresh egg instead of the commercially dried egg generally used for this diet, and results for a small group of littermates fed the diet of egg yolk supplemented with salt mixture. The results for rats fed diets containing egg white with HVO as the fat (SPW 8 HVO and SPEW) were similar to those obtained for comparable rats fed the semipurified diet (tables 22, 23, 25, 26). The results with SPE-fresh egg were similar to those obtained on the usual SPE diet. Among the animals that were losing weight, large kidneys were a frequent finding with all of the diets, but those from rats fed SPE diet tended to be the largest. Kidneys and livers were generally smaller when the diet consisted of 100 percent egg yolk than when it contained a smaller amount of egg yolk. The favorable response to supplementation of Y100 with 3 percent salt mixture discussed under the histological findings was also apparent in the relatively small kidneys and livers in these rats.

STOCK AND SPE DIETS REVERSED AT 250 DAYS.—In table 35 are summarized data for kidney and liver weights when the diet was changed from stock to SPE or the reverse at 250 days of age. The kidney and liver weights of the 250-day-old rats were characteristic of those generally observed with these two diets. No difference in the size of these organs was observed whether the rats ate a constant diet throughout life or had their diets reversed. It should be remembered, however, that when the diets were reversed, the age at death was considerably greater than when either diet was fed continuously throughout life.

BHE AND WISTAR RATS FED SP 8 HVO AND SPE DIETS.—Differences in response to diet of two strains of rats are seen in table 36, which summarizes data for kidney and liver weights of

TABLE 33.—*Kidney and liver weights of rats losing weight on SP 8 HVO diet modified to contain the protein and fat level of SPE diet*

Diet	Rats	Average age	Average weight loss	Kidney weight		Liver weight	
				Average	Range	Average	Range
	Number	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
SP 8 HVO	17	679	164	3. 46	1. 57–7. 80	16. 1	10. 8–22. 4
SPa 16 HVO	18	674	175	3. 78	1. 57–7. 92	17. 0	12. 4–24. 9
SPb 8 HVO	9	632	118	2. 94	1. 46–5. 07	[1] 16. 3 (8)	11. 2–21. 6

[1] Number in parentheses indicates number of animals when differing from that in column 1.

TABLE 34.—*Kidney and liver weights of rats fed various diets containing egg, egg yolk, or egg white*

Condition, weight status, and diet	Rats	Average age	Average weight loss	Kidney weight		Liver weight	
				Average	Range	Average	Range
Fasted rats							
Maintaining weight:	*Number*	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
SPW	6	550	0	1. 73	1. 61– 2. 18	13. 6	11. 3–18. 0
Fasted and nonfasted rats							
Losing less than 100 grams:							
E100	8	558	48	4. 19	1. 87–10. 8	[1] 29. 7 (6)	16. 8–42. 6
Y100	16	390	59	2. 29	. 93– 5. 99	15. 1 (15)	11. 0–24. 3
Losing more than 100 grams:							
SPE—fresh egg	7	492	167	7. 87	4. 06–12. 6	23. 1 (6)	15. 8–40. 1
SPEW	8	608	184	4. 30	1. 59– 8. 28	19. 4 (5)	12. 7–29. 9
SPEY	9	480	168	5. 89	3. 45– 8. 13	19. 1 (7)	15. 2–26. 0
E100	16	542	156	4. 80	1. 52– 8. 68	16. 3	12. 0–23. 5
Y100	3	445	152	3. 36	1. 53– 5. 74	15. 3	14. 1–17. 1
Fasted rats							
Littermates:							
SPE	5	428	66	3. 88	2. 02– 7. 25	21. 5	14. 0–25. 6
Y100	5	425	35	2. 71	1. 63– 4. 46	17. 3	13. 9–24. 3
Y97+salt mixture, 3.0%	5	430	5	1. 68	1. 55– 1. 75	14. 5	13. 2–16. 5

[1] Numbers in parentheses indicate number of animals when differing from that listed in column 1.

TABLE 35.—*Kidney and liver weights of rats fed stock or SPE diets reversed at 250 days*

Condition and diet of rats	Rats	Age	Weight loss	Kidney weight	Liver weight
Sacrificed at approximately 250 days on—	*Number*	*Days*	*Grams*	*Grams*	*Grams*
Stock	3	250	0	2. 07	17. 0
SPE	3	249	0	2. 74	26. 4
Continued on—					
Stock	3	590	195	4. 25	18. 5
SPE	3	473	104	4. 17	22. 2
Diet reversal:					
Stock changed to SPE at 250 days	4	686	109	3. 94	22. 3
SPE changed to stock at 250 days	4	624	101	3. 48	21. 7

BHE and Wistar rats fed SP 8 HVO and SPE diets. The kidneys were generally small even with SPE diet, in marked contrast to the large kidneys from comparable BHE rats. Kidneys from only two rats of the Wistar strain exceeded 4 grams, one from a rat fed SP 8 HVO diet and one fed SPE diet. For both diets, livers tended to be smaller in Wistar than in comparable BHE

TABLE 36.—*Kidney and liver weights in two strains of rats at terminal age from parents fed two stock diets, with young fed SP 8 HVO and SPE diets*

Strain and diet	Rats	Average age	Average weight loss	Kidney weight		Liver weight	
				Average	Range	Average	Range
BHE rats							
Parents fed BHE stock diet:							
Offspring fed—	*Number*	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
SP 8 HVO	11	571	136	3. 76	1. 70– 7. 80	16. 9	10. 0–24. 7
SPE	14	438	151	8. 24	2. 68–13. 3	21. 4	15. 3–38. 7
Wistar rats							
Parents fed Wistar stock diet:							
Offspring fed—							
SP 8 HVO	10	739	108	2. 00	1. 34– 4. 11	[1] 13. 7 (8)	9. 0–24. 6
SPE	9	633	80	2. 35	1. 60– 4. 34	18. 9	15. 0–21. 9
BHE rats							
Parents fed Wistar stock diet:							
Offspring fed—							
Wistar stock	5	886		1. 22	1. 12– 1. 40	10. 2	9. 6–12. 4
SP 8 HVO	9	703	75	2. 41	1. 04– 4. 07	12. 2	10. 0–15. 7
SPE	10	435	85	5. 14	. 99–10. 4	[1] 21. 6 (9)	13. 7–34. 7

[1] Numbers in parentheses indicate the number of animals when differing from that listed in column 1.

rats. The livers from Wistar rats fed SPE diet tended to be larger than those from the same strain fed SP 8 HVO diet.

BHE rats from parents raised on the stock diet of the Wistar rats showed differences in kidney and liver weights with SP 8 HVO and SPE diets that were similar to those found when the parents were raised on regular stock diet. The results agree with the histological findings, and provide further evidence that the dietary history of stock Wistar rats was not responsible for differences in the response of BHE and Wistar rats to diet.

DISCUSSION.—Much of the information in the literature on kidney weights in the rat has been based on studies during the period of rapid growth or for the young adult rat. There appears to be a tendency for the right kidney to be somewhat larger than the left kidney (*4, 123*), although the differences observed were small (3 to 5 percent). There has been general agreement that the weight of this organ is closely related to body weight, but the value for this relationship has been found to vary with the strain of animal under investigation. The values tabulated in Donaldson's reference tables for Wistar rats (*50*) were obtained by using the formula of Hatai (*86*) relating kidney to body weight. For male rats weighing 450 grams, the value recorded for the weight of two kidneys was 3.5 grams or 0.78 percent of body weight. Freudenberger (*70*) has reported kidney weights with a similar relationship to body weight for male Wistar rats 1 year old. For comparable rats of the Long-Evans hybrid strain of Norway rats, the average kidney weight represented a somewhat smaller percentage of the body weight, 0.69. Addis and Gray (*4*), using a log log relation between body and kidney weight, have developed an equation for the Slonaker strain of rats and have presented standards for kidney weights of rats with body weights up to 400 grams. On the basis of the standards for the Slonaker strain, animals weighing 400 grams would have kidneys weighing 0.54 percent of their body weight.

The results reported in this publication provide no information on changes in kidney size in the young growing rat. For fasted adult rats under 300 days of age, kidney weights, when considered in relation to body weight, were within the range reported by other investigators.

The influence of feeding various dietary combinations throughout life on the kidney size of the rat has received relatively little attention to date. Many of the available reports relating diet to kidney size have dealt with short-time feeding studies, often under extreme conditions producing acute changes. The emphasis has been chiefly on the influence of dietary protein on kidney size, and there has been general agreement that kidney size is increased with increasing levels of protein.

Osborne, Mendel, Park, and Winternitz (*150*) observed an invariable increment in the size of the kidney when the casein content of the diet exceeded 50 percent. This gain in kidney weight often amounted to 50 percent or more above the normal weight. When the casein level exceeded 75 percent, unmistakable evidence of kidney enlargement was found within 8 days. Equally striking changes were observed when rats were fed a diet containing 80 percent "meat residue."

McCay, Maynard, Sperling, and Osgood (*121*) reported a correlation of kidney weight with level of protein when diets containing 8, 14, and 20 percent casein were fed. These dietary regimens were initiated during the latter half of life and did not involve excessively high levels of protein. The kidney size reflected dietary protein level at death in spite of the wasting that accompanied the last diseases of old age. Addis, Lippman, Lew, and others (*5*) found that kidney size increased when the level of egg, liver, wheat germ, or casein was increased from 10 to 60 percent, and that the extent of the enlargement depended upon the source of protein. Smith and Moise (*174*) reported that, after removal of one kidney, the remaining kidney became enlarged and that the increase was proportional to the level of dietary casein.

Agreement with regard to the cause of increased kidney size as the result of feeding high levels of protein has been less general. Addis, Lippman, Lew, and others (*5*) suggested that the differential effect of dietary protein upon kidney size was not due to differences in its inherent nutritive value for the kidney but was secondary to the work load imposed upon the kidney by urea excretion. Other investigators (*138*, *150*) conclude that the load on the kidney from increased urea excretion is not responsible for kidney enlargement on the basis of the results of feeding high levels of urea.

In general, the data available on the size of liver of rats are similar in nature to those reported for the kidney, and have also indicated a close relationship between liver and body weight. Donaldson (*50*) has reported a value of 18.7 grams (4.2 percent of body weight) for the liver from fasted male rats weighing 450 grams. Liver weights of 12.1 and 16.4 grams have been reported by Freudenberger (*70*) for 1-year-old male rats of the Wistar and Long-Evans strain, respectively. The Wistar rats weighed 340 grams; the Long-Evans rats, 458 grams. No strain differences were apparent, since the livers of both strains represented the same percentage of body weight (3.6 percent). Addis and Gray (*4*) reported still smaller liver weights for the Slonaker strain, with the values representing 3.0 to 3.2 percent of the body weight in animals weighing between 360 and 410 grams. These last-named investigators suggested that a possible explanation for the differences observed may lie in the higher protein level of the diets fed the rats with the larger livers. For rats 1 to 2 years of age, Yiengst, Barrows, and Shock (*191*) observed liver weights for the McCollum strain of rats of 13.3 and 13.0 grams, representing 2.8 and 3.0 percent of the body weight, respectively. No significant change with age was observed.

Several investigators reported results indicating that liver weight may be influenced by diet without comparable changes in body weight. Blatherwick, Medlar, Bradshaw, and others (*31*) reported variations in this percentage from 2.8 to 5.2. Addis, Lippman, Lew, and others (*5*) obtained an increase in liver weight of approximately 10 percent, with no comparable increase in body weight, when rats were fed 60 percent wheat germ, liver, or egg powder for a period of 20 days. At the 10-percent level, diets produced no change in liver weight. Casein was without influence at either the 10- or 60-percent level. McCay, Maynard, Sperling, and Osgood (*121*) reported a 10-percent increase, not accompanied by a comparable increase in body weight, when the level of casein in the diet was increased from 8 to 20 percent and the rats were fed the experimental diets from middle age until death. Large livers have been reported in rats fed diets containing high levels of egg (*156*) or liver (*31*).

Even with the stock diet, BHE rats were relatively large animals, but in spite of their large body weight the livers of the fasted rats were generally smaller than those reported by many investigators. The livers from fasted rats on the stock diet were similar in size and in percentage of body weight to those reported by Yiengst, Barrows, and Shock (*191*). Liver weights of 10 grams reported by Reussner and Thiessen (*156*) for 9-month-old rats fed a diet of cereal, milk, and sugar or milk alone were similar to those obtained with BHE rats of comparable size fed most of the experimental diets. The average liver weight of 15 grams observed by these investigators for rats fed a bacon and egg diet containing 22 percent egg was similar to that obtained for BHE rats fed SPE diet, when considered as percent of body weight.

Harrison (*85*) reported relatively small differences between the liver weight of young fasted and nonfasted rats fed stock ration. A search of the literature has failed to reveal investigations showing differences in the liver weights of fasted and nonfasted rats of the magnitude observed with BHE rats fed the semipurified diet or its modifications.

Summary.—A kidney weighing less than 1.8 grams was generally normal in appearance. Degenerative changes were usually apparent when the kidney weight exceeded 1.8 grams, and cystic and glomerular damage as well as hyalin casts were usually observed when the weight exceeded 3 grams.

In *fasted rats* that were maintaining their weight, differences in kidney or liver size in relation to age and to diet were frequently associated with parallel differences in body weight. Large kidneys and livers were observed in some of the older rats fed all diets. Kidneys tended to be large in rats 300 days and older fed SPE diet, regardless of body weight. Large livers were found even in young rats 200 to 300 days of age that were fed this diet. Although there was a tendency for kidney or liver weight to parallel body weight, a somewhat closer relationship was observed between kidney and liver weights, at least in rats fed stock or the semipurified diet.

In *nonfasted rats* that were maintaining weight, both kidneys and livers were larger than those in comparable fasted rats. The difference between the size of these organs in fasted and nonfasted rats varied with diet and age.

In *moribund rats*, enlarged kidneys were a frequent finding on all diets, particularly when weight loss exceeded 100 grams. The extent of the enlargement observed varied with diet. Some extremely large kidneys were obtained from rats fed SPE and SPPB diets. The marked increase in kidney weight that occurred with increasing loss of body weight was not accompanied by a comparable increase in liver weight.

The kidneys and livers from *moribund Wistar* rats were generally small, even on SPE diet, in contrast to the results with comparable BHE rats fed this diet.

Adrenal weight

Rats maintaining weight on stock, SP 8 HVO, and SPE diets.—In table 37 are summarized data for the weights of the adrenals from rats in different age groups that had been fed stock, SP 8 HVO, or SPE diets, and were maintaining weight at the time of sacrifice. No significant differences were observed between the adrenal weights of fasted and nonfasted rats, and the results recorded are the combined data for both groups of animals.

The average adrenal weight for 98 rats fed the *stock diet* was 19.8 mg., with a wide range of values from 12.4 to 33.5. No differences were noted with age, either in the average values or in the distribution within groups, except for a tendency to larger adrenals in the small group of rats 700 days and older.

On *SP 8 HVO diet*, the average adrenal weight of 20.2 mg. for 64 rats was similar to that for rats fed the stock diet, and again, age appeared to have little effect on the size of this organ. The body weight of these rats differed on the average by 146 grams so that the relationship of the adrenal to body weight for these two groups of animals differed significantly.

On *SPE diet*, adrenals tended to be larger than those from rats of comparable age fed stock or SP 8 HVO diets. The largest average adrenal weight was observed for rats 300 to 399 days of age; seven of these rats had adrenals exceeding 30 mg. This age group corresponds to the group previously shown to have very large and damaged kidneys.

In table 38 are summarized data for adrenal weights separated according to body weight. Average adrenal weights tended to increase with body weight on all three diets. The wide range of values within groups and the relatively low correlation coefficients of 0.35, 0.55, and 0.38 for stock, SP 8 HVO, and SPE diets, respectively, indicate that factors other than body weight were also influencing the weight of this organ.

Rats losing weight on stock, SP 8 HVO, and SPE diets.—Data for the size of adrenals from sick or moribund rats fed stock, SP 8 HVO, or SPE diets are summarized in table 39. The age groups available differ, depending upon the influence of these diets on survival. Adrenal size was influenced appreciably by the extent of weight loss

Table 37.—*Adrenal weights of rats maintaining weight at different ages on stock, SP 8 HVO, and SPE diets*

Diet and age of rats (days)	Rats	Average age	Average body weight	Adrenal weight		Adrenals weighing—			
				Average	Range	Less than 15.0 mg.	15.0 to 19.9 mg.	20.0 to 29.9 mg.	30.0 mg. and over
Stock:	*Number*	*Days*	*Grams*	*Mg.*	*Mg.*	*Percent*	*Percent*	*Percent*	*Percent*
Less than 200	19	151	390	18.9	12.4–23.6	11	53	37	0
200 to 299	24	249	454	19.2	14.0–24.5	8	50	42	0
300 to 399	13	381	452	19.7	13.6–24.4	8	46	46	0
400 to 499	13	449	475	19.6	15.4–24.6	0	46	54	0
500 to 599	12	538	493	20.1	13.7–25.8	17	25	58	0
600 to 699	12	652	468	20.3	14.5–26.0	8	58	33	0
700 and over	5	844	468	25.6	17.9–33.5	0	20	60	20
Average—all	98	389	451	19.8	12.4–33.5	8	46	45	1
SP 8 HVO:									
Less than 200	5	154	524	19.2	14.3–21.2	20	20	60	0
200 to 299	12	250	518	18.0	13.8–24.3	8	67	25	0
300 to 399	31	337	614	21.0	14.3–32.7	3	45	48	3
400 to 499	7	457	609	19.9	11.1–27.4	14	29	57	0
500 to 599	9	550	677	21.0	15.4–26.0	0	33	67	0
Average—all	64	349	597	20.2	11.1–32.7	6	44	48	2
SPE:									
Less than 200	5	154	524	20.8	19.8–21.8	0	20	80	0
200 to 299	25	270	599	20.4	14.8–27.7	4	40	56	0
300 to 399	34	336	616	27.0	17.1–55.3	0	6	74	21
400 to 499	14	455	621	23.3	17.1–29.0	0	14	86	0
500 to 599	9	527	637	23.0	16.9–28.4	0	33	67	0
Average—all	87	345	609	23.7	14.8–55.3	1	21	71	7

TABLE 38.—*Adrenal weight in relation to body weight of rats fed stock, SP 8 HVO, and SPE diets*

Diet and body weight of rats (grams)	Rats	Average body weight	Adrenal weight		Adrenal to body weight	Adrenals weighing—			
			Average	Range		Less than 15.0 mg.	15.0 to 19.9 mg.	20.0 to 29.9 mg.	30.0 mg. and over
Stock:	*Number*	*Grams*	*Mg.*	*Mg.*	*Mg./100 g.*	*Percent*	*Percent*	*Percent*	*Percent*
300 to 399	16	372	17. 7	12. 4–22. 6	4. 8	19	62	19	0
400 to 449	26	430	10. 6	13. 7–29. 3	4. 6	8	46	46	0
450 to 499	43	471	20. 4	14. 6–25. 9	4. 3	5	40	56	0
500 and over	12	547	21. 7	16. 3–33. 5	4. 0	0	42	50	8
SP 8 HVO:									
400 to 499	10	466	16. 7	11. 1–20. 1	3. 6	30	60	10	0
500 to 599	20	547	18. 8	14. 3–24. 5	3. 4	5	60	35	0
600 to 699	26	646	21. 5	16. 3–32. 7	3. 3	0	31	65	4
700 and over	8	730	23. 2	18. 4–28. 9	3. 2	0	25	75	0
SPE:									
400 to 499	5	471	19. 1	15. 2–24. 9	4. 1	0	60	40	0
500 to 599	43	560	22. 4	14. 8–47. 8	4. 0	2	21	74	2
600 to 699	30	641	25. 3	17. 1–35. 6	4. 0	0	10	80	10
700 to 799	8	736	26. 6	17. 1–55. 3	3. 6	0	38	38	25

TABLE 39.—*Adrenal weights of rats losing weight at different ages on stock, SP 8 HVO, and SPE diets*

Weight loss, diet, and age of rats (days)	Rats	Average age	Average weight loss	Adrenal weight		Adrenals weighing—			
				Average	Range	Less than 20.0 mg.	20.0 to 29.9 mg.	30.0 to 39.9 mg.	40.0 mg. and over
Weight loss less than 100 grams:									
Stock:	*Number*	*Days*	*Grams*	*Mg.*	*Mg.*	*Percent*	*Percent*	*Percent*	*Percent*
300 to 699	5	592	56	21. 7	18. 4–26. 7	40	60	0	0
700 to 799	4	763	65	25. 3	15. 1–24. 7	25	50	25	0
800 and over	5	853	66	30. 0	26. 5–36. 3	0	60	40	0
SP 8 HVO:									
Less than 400	7	245	34	20. 5	17. 1–32. 6	71	14	14	0
400 to 599	12	491	61	23. 8	14. 4–41–2	33	50	8	8
600 to 799	9	669	46	25. 8	19. 5–35. 9	11	78	11	0
SPE:									
Less than 300	9	185	23	23. 5	17. 6–33. 5	33	44	22	0
300 to 499	39	401	51	28. 9	17. 4–44. 5	3	62	28	8
500 to 699	17	590	52	32. 8	25. 2–63. 6	0	41	47	12
Weight loss more than 100 grams:									
Stock:									
300 to 699	8	521	125	37. 1	26. 9–53. 1	0	25	50	25
700 to 799	4	776	193	38. 0	23. 8–47. 3	0	25	25	50
800 and over	2	808	161	45. 2	33. 6, 56. 7	0	0	50	50
SP 8 HVO:									
Less than 500	5	422	155	32. 7	26. 3–39. 3	0	40	60	0
500 to 699	15	600	169	35. 7	16. 8–47. 5	13	7	40	40
700 and over	9	829	205	31. 8	18. 0–45. 0	11	33	33	22
SPE:									
Less than 400	20	352	150	39. 4	28. 5–58. 4	0	10	55	35
400 to 499	23	443	164	36. 5	18. 0–56. 7	4	17	39	39
500 to 699	32	583	160	35. 3	24. 3–57. 7	0	28	47	25

before sacrifice, and the results have been separated accordingly. Even when weight loss was less than 100 grams, adrenal weights tended to be larger than those found in animals that were maintaining weight. Adrenals from rats fed SPE diet were consistently larger than those from rats of comparable age fed stock or SP 8 HVO diets. Adrenals tended to increase in size with age, in contrast to the results for rats that were maintaining weight. When weight loss exceeded 100 grams, adrenals were large for all age groups and the influence of diet was no longer apparent.

Adrenal weight and kidney damage.—A tendency for large adrenals to be associated with damaged kidneys is seen in table 40. When adrenals weighed less than 25 mg. there was little evidence of kidney damage in rats that were maintaining weight on stock or SP 8 HVO diets. In the few rats with adrenals exceeding 25 mg., some kidney damage was generally observed.

In rats fed SPE diet, kidney damage was apparent in several rats with adrenals weighing less than 25 mg., and the extent of the damage tended to increase with the size of this gland. In rats that were losing weight at the time of sacrifice, the extent of the degenerative changes in the kidneys tended to parallel adrenal size.

Large adrenals were observed occasionally on all diets, accompanied by kidneys showing little or no damage. These results suggest that adrenal weights may be reflecting the health of the rat and that the apparent parallelism with kidney damage may be the result of the high incidence of renal disease in these rats.

Rats fed SPM, SPB, and SPPB diets.—Limited data on adrenal size in rats *maintaining weight* on SPM, SPB, or SPPB diets are summarized in table 41. The adrenals were similar in size with all three diets and did not differ appreciably from those in rats of comparable body weight fed SP 8 HVO diet. They represented 3.3 mg./100 g. of the body weight, a value considerably lower than that obtained for the smaller rats fed the stock diet (table 38).

In *moribund rats* (table 42), adrenals tended to be large even when weight loss was less than 100 grams. Seventy percent of the adrenals from rats 500 days and older fed SPB or SPPB diets exceeded 30 mg. When weight loss was more than 100 grams, adrenals were large, regardless of age or diet, except for the tendency to smaller adrenals in the rats fed SPM diet that were less than 500 days old.

Rats fed other experimental diets.—In table 43 are summarized data for adrenal weights of rats fed all other experimental diets. The results are for moribund rats except for a group fed SPW 8 HVO diet, in which egg white replaced the casein and lactalbumin of the semipurified diet, and a group fed egg yolk with or without mineral supplementation. Because of the limited amount of data for most of these diets, the results are recorded as average values without separation by weight loss. There was considerable variation in

Table 40.—*Adrenal weight in relation to extent and kind of kidney damage in rats maintaining or losing weight on stock, SP 8 HVO, or SPE diets*

Weight status, diet, and adrenal weight (mg.)	Rats	Average adrenal weight	Histological rating for kidney damage		
			Hyalin	Cystic	Glomerular
Rats maintaining weight:					
Stock:	*Number*	*Mg.*	*Score*	*Score*	*Score*
Less than 20.0	46	17. 2	0. 3	0. 1	0. 1
20.0 to 24.9	31	22. 4	. 4	. 1	. 1
25.0 and over	6	26. 4	1. 5	1. 0	1. 5
SP 8 HVO:					
Less than 20.0	22	16. 6	. 2	0	0
20.0 to 24.9	16	21. 9	. 5	. 2	. 2
25.0 and over	2	26. 7	1. 0	0	0
SPE:					
Less than 20.0	16	18. 3	. 8	0	0
20.0 to 24.9	32	22. 6	1. 2	. 2	0
25.0 and over	15	27. 1	1. 6	. 5	. 3
Rats losing weight:					
Stock:					
Less than 20.0	3	17. 6	. 7	. 3	0
20.0 to 24.9	4	22. 8	1. 0	. 8	. 8
25.0 to 29.9	7	27. 7	2. 3	. 7	1. 1
30.0 to 39.9	8	34. 9	2. 0	2. 0	2. 4
40.0 and over	5	48. 6	2. 6	2. 2	2. 8
SP 8 HVO:					
Less than 20.0	12	17. 7	. 6	. 3	. 1
20.0 to 24.9	8	22. 1	. 8	0	0
25.0 to 29.9	12	27. 6	1. 3	. 8	. 6
30.0 to 39.9	15	34. 1	2. 2	1. 6	1. 5
40.0 and over	9	43. 9	2. 4	1. 9	1. 6
SPE:					
Less than 20.0	3	17. 8	1. 7	1. 0	. 7
20.0 to 24.9	15	22. 5	1. 5	1. 0	. 7
25.0 to 29.9	33	27. 7	2. 3	1. 9	1. 3
30.0 to 39.9	56	34. 2	2. 3	2. 9	1. 8
40.0 and over	28	46. 1	2. 4	2. 9	2. 1

TABLE 41.—*Adrenal weights of rats maintaining weight at different ages on SPM, SPB, and SPPB diets*

Diet and age of rats (days)	Rats	Average age	Average body weight	Adrenal weight		Adrenals weighing—			
				Average	Range	Less than 15.0 mg.	15.0 to 19.9 mg.	20.0 to 29.9 mg.	30.0 mg. and over
SPM:	*Number*	*Days*	*Grams*	*Mg.*	*Mg.*	*Percent*	*Percent*	*Percent*	*Percent*
Less than 300	13	214	553	17. 4	7. 0–26. 3	23	38	38	0
300 to 499	6	399	672	21. 4	19. 0–24. 0	0	33	67	0
500 to 599	2	550	624	22. 4	19. 6, 25. 3	0	50	50	0
SPB:									
Less than 300	13	214	520	16. 9	11. 5–20. 3	31	38	31	0
300 to 499	7	413	596	21. 2	16. 1–30. 8	0	43	43	14
500 to 599	3	549	673	22. 2	19. 9–23. 6	0	33	67	0
SPPB:									
Less than 300	14	217	535	18. 7	5. 7–28. 3	21	36	43	0
300 to 499	5	412	644	20. 8	14. 2–26. 7	20	20	60	0
500 to 599	6	550	728	23. 7	18. 6–29. 7	0	17	83	0

TABLE 42.—*Adrenal weights of rats losing weight in two age groups fed SPM, SPB, and SPPB diets*

Weight loss, age of rats (days), and diet	Rats	Average age	Average weight loss	Adrenal weight		Adrenals weighing—				
				Average	Range	Less than 15.0 mg.	15.0 to 19.9 mg.	20.0 to 29.9 mg.	30.0 to 39.9 mg.	40.0 mg. and over
Weight loss less than 100 grams:										
Less than 500 days:	*Number*	*Days*	*Grams*	*Mg.*	*Mg.*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
SPM	11	358	49	23. 7	17. 7–36. 8	0	36	45	18	0
SPB	9	308	46	24. 3	15. 2–32. 1	0	22	56	22	0
SPPB	22	338	42	26. 5	12. 8–50. 2	14	9	55	14	9
500 days and older:										
SPM	7	585	30	28. 9	17. 0–39. 7	0	14	29	57	0
SPB	7	671	53	34. 6	22. 9–47. 2	0	0	29	29	43
SPPB	10	625	66	33. 4	21. 1–45. 8	0	0	30	40	30
Weight loss more than 100 grams:										
Less than 500 days:										
SPM	6	421	144	25. 1	16. 3–35. 7	0	33	50	16	0
SPB	5	450	186	36. 5	25. 5–48. 5	0	0	40	20	40
SPPB	5	397	192	36. 9	28. 9–52. 2	0	0	40	20	40
500 days and older:										
SPM	7	775	207	34. 2	26. 8–44. 9	0	0	29	57	14
SPB	13	616	186	36. 4	16. 6–53. 8	0	8	15	46	31
SPPB	8	568	168	34. 3	24. 9–44. 4	0	0	38	38	25

the loss in body weight before sacrifice and many of the differences observed seem to be related to the extent of this loss.

Adrenals tended to be relatively small in rats fed SPE diet supplemented with vitamin B_6 with or without choline, despite the extensive weight loss of most of these rats. Exceedingly large adrenals were observed in rats consuming 100 percent egg yolk or whole egg even before weight loss became excessive. Supplementation of egg yolk with 3 percent salt mixture resulted in a reduction in adrenal size from an average of 33.7 mg. to 23.0 mg. Wistar rats, except for one with an exceedingly large adrenal (91.7 mg.), tended to have smaller adrenals than comparable BHE rats. Wistar rats fed SPE diet had generally larger adrenals than did those rats fed SP 8 HVO diet, thus showing a dietary response similar to that observed with BHE rats.

DISCUSSION.—Although data on the weight of rat adrenals have been reported by several investigators, relatively little information is available for animals comparable in size to the experimental animals considered in this publication. As the result of domestication of the wild Norway rat (*160*), a marked decrease in adrenal weight has occurred. Donaldson (*50*) reported 49 mg. as the weight of two adrenals for 340-gram rats of the Wistar strain. The value recorded by Freudenberger (*70*) for animals of comparable size is

TABLE 43.—*Adrenal weights of rats fed other experimental diets*

Strain and diet	Rats	Average age	Average weight loss	Adrenal weight	
				Average	Range
BHE rats					
SPE supplemented with—	*Number*	*Days*	*Grams*	*Mg.*	*Mg.*
Choline, 0.5%	22	474	136	33. 9	22. 7–53. 3
B_{12}, 0.01 mg./100 gm	8	463	131	32. 5	21. 5–39. 9
Choline, 0.5% + B_{12}, 0.01 mg./100 gm	10	434	114	32. 6	18. 6–47. 2
B_6, 0.5 mg./100 gm	7	465	180	30. 1	23. 6–35. 5
Choline, 0.5% + B_6, 0.5 mg./100 gm	9	412	128	28. 0	21. 8–40. 7
Choline, 0.5%+B_{12}, 0.01 mg./100 gm.+B_6, 0.5 mg./100 gm	10	430	138	32. 1	21. 7–42. 9
Cholesterol, 0.46%	7	460	155	32. 4	24. 0–40. 2
Cholesterol, 1.38%	9	406	142	33. 5	25. 0–40. 9
Ascorbic acid, 0.2%	8	436	129	34. 0	19–9–44. 1
Ascorbic acid, 0.2%+cholesterol, 0.46%	9	434	183	36. 9	30. 2–58. 6
SP 16 HVO	4	586	77	30. 8	20. 6–42. 1
SP 8 lard	15	553	98	34. 0	17. 9–60. 8
SP 16 lard	7	514	109	28. 9	23. 0–35. 2
SP 8 butter	6	545	75	26. 3	17. 3–40. 9
SP 16 butter	10	503	66	29. 4	11. 7–60. 3
SPa 16 HVO	17	637	172	32. 8	19. 0–44. 2
SPb 8 HVO	9	632	118	31. 8	22. 0–47. 8
SPE (fresh egg)	6	483	166	36. 3	18. 0–47. 7
SPEW	6	530	155	33. 2	18. 6–47. 0
SPEY	7	473	178	44. 5	32. 7–53. 3
SPW 8 HVO	6	550	0	15. 0	11. 4–19. 3
E100	21	541	129	37. 8	23. 1–46. 0
Y100	19	400	73	35. 4	20. 2–46. 1
Littermates fed—					
SPE	5	428	66	27. 6	23. 2–32. 4
Y100	5	425	35	33. 7	24. 4–50. 1
Y97+salt mixture, 3.0%	5	430	5	23. 0	18. 8–26. 6
Diet reversal:					
Sacrificed at approximately 250 days:					
Stock	3	250	0	22. 9	17. 5–27. 4
SPE	3	249	0	23. 0	20. 5–26. 2
Continued on—					
Stock	2	574	207	25. 6	25. 3, 26. 0
SPE	2	396	133	30. 5	22. 8, 38. 2
Reversed at 250 days:					
Stock changed to SPE	4	686	109	33. 4	23. 2–40. 2
SPE changed to stock	3	577	128	30. 3	24. 7–40. 2
Wistar rats					
SP 8 HVO	9	727	106	24. 2	15. 9–41. 1
SPE	9	664	80	35. 8	19. 0–91. 7
BHE rats from parents fed Wistar stock diet					
SP 8 HVO	9	703	74	31. 1	19. 1–48. 3
SPE	9	456	81	32. 3	17. 8–46. 0

somewhat smaller, 31 mg. For male rats of the Slonaker strain weighing 340 grams, Addis and Gray (*4*) obtained a value of 37 mg. According to Freudenberger (*70*), there was little evidence for strain differences between Wistar and Long-Evans rats when weight differences between strains were considered. Yeakel (*190*) compared adrenal weights of rats 700 days of age and older with those of young rats, and reported consistently heavier adrenals in old rats whether considered in terms of absolute weight or in relation to body weight.

Several reports dealing with the influence of diet on adrenal size have also appeared. High-protein diets have resulted in larger adrenal weights than were obtained with high-carbohydrate diets (*32, 97, 181*). The response to protein from the same source may vary, depending upon the specific combination of dietary ingredients. Fetter and Neidle (*60*) and Ingle, Ginther, and Nezamis (*98*) observed increased adrenal weights on high-fat diets, although the differences observed by Ingle were small.

There is general agreement that many kinds of shock or stress result in increased adrenal size. Tepperman, Engel, and Long (*180*) and Sayers

(*169*) have reviewed the many factors that may result in adrenal enlargement. Infection generally causes marked hypertrophy. A long-continued stress ending in death results in marked hypertrophy and hyperplasia, with the degree of hypertrophy proportioned to the time which elapses between the onset of stress and death.

SUMMARY.—In rats that were *maintaining weight* when sacrificed, adrenal weights showed relatively little variation with diet. The largest adrenals were found in rats on the diet containing 25 percent egg or consisting of 100 percent egg yolk. Age also exerted little influence on adrenal size. The relation of adrenal weight to body weight varied significantly with diet. For any one diet, adrenal size tended to parallel body weight but the relatively low correlation coefficients for this relationship indicated that the weight of this organ was reflecting other factors as well.

In rats that were *losing weight*, adrenals tended to be large and no differences due to diet were apparent when weight loss exceeded 100 grams. When extensive kidney damage was present, adrenals were generally large. The occasional occurrence of large adrenals in rats with apparently normal kidneys, however, suggests that adrenal weights may be reflecting the general health of the animal rather than a direct relation to kidney damage.

A comparison of the results of moribund Wistar and BHE rats suggests a hereditary difference in the response to stress, but provides no information concerning possible differences in the weight of this gland in normal healthy animals.

Thyroid weight

RATS MAINTAINING WEIGHT ON STOCK, SP 8 HVO, AND SPE DIETS.—Data for the thyroid weights of rats maintaining weight on stock, SP 8 HVO, or SPE diets are summarized in table 44. The results for fasted and nonfasted rats have been combined and the thyroid weights in all cases include the parathyroids. On *stock diet*, thyroid weights tended to increase with age up to 700 days, but the differences were small considering the wide range of values observed for any one age group. No large thyroids were observed in animals over 700 days old, in contrast to the relatively large adrenals from these same rats. For rats fed *SP 8 HVO diet*, age appeared to be without influence. The average of 11.8 mg. for the thyroid weights of animals fed this diet was similar to the average of 11.1 for stock rats, in spite of the large difference in the body weights of these two groups. On *SPE diet* also, age appeared to exert no consistent influence on the size of this gland. The average weight of 16.2 mg. for the thyroids from rats fed SPE diet was significantly more than the average for rats fed either stock or SP 8 HVO diet ($P<0.01$).

In table 45 are summarized data for thyroid weights in relation to body weight. The range of values for any one group was wide and correlation coefficients were relatively low—0.42, 0.46, and 0.31 for stock, SP 8 HVO, and SPE diets, respectively. There was, however, a consistent tendency for thyroid weights to increase with body weight on all three diets, as evidenced

TABLE 44.—*Thyroid weights of rats maintaining weight at different ages on stock, SP 8 HVO, and SPE diets*

Diet and age of rats (days)	Rats	Average age	Average body weight	Thyroid weight		Thyroids weighing—			
				Average	Range	Less than 10.0 mg.	10.0 to 14.9 mg.	15.0 to 19.9 mg.	20.0 mg. and over
Stock:	*Number*	*Days*	*Grams*	*Mg.*	*Mg.*	*Percent*	*Percent*	*Percent*	*Percent*
Less than 200	19	151	390	9.7	6.5–13.3	63	37	0	0
200 to 299	24	249	454	10.2	5.8–15.7	42	54	4	0
300 to 399	13	381	452	11.2	5.6–15.6	31	54	15	0
400 to 499	13	449	475	11.9	7.4–21.7	23	62	8	8
500 to 599	12	538	493	12.6	9.2–17.0	8	75	17	0
600 to 699	12	652	468	12.8	6.0–16.6	25	42	33	0
700 and over	8	844	468	10.4	7.1–14.9	40	60	0	0
SP 8 HVO:									
Less than 200	5	154	524	10.5	8.5–12.0	40	60	0	0
200 to 299	12	250	518	10.2	6.6–18.7	58	33	8	0
300 to 399	31	337	614	12.4	6.7–17.5	13	74	13	0
400 to 499	7	457	609	10.1	6.9–15.7	71	14	14	0
500 to 599	9	550	677	13.8	7.8–19.9	11	44	44	0
SPE:									
Less than 200	5	154	524	14.5	11.9–17.5	0	60	40	0
200 to 299	25	270	599	15.0	9.1–20.8	4	44	48	4
300 to 399	34	336	616	17.8	10.6–30.4	0	29	47	24
400 to 499	14	455	621	13.9	6.1–20.4	21	50	21	7
500 to 599	9	527	637	18.1	12.3–23.6	0	11	56	33

by the increased proportion of large thyroids among the heavy animals.

Rats losing weight on stock, SP 8 HVO, and SPE diets.—The thyroid weights of sick or moribund rats fed stock, SP 8 HVO, or SPE diet are summarized in table 46, with the results for rats that lost less than 100 grams before sacrifice separated from those for rats with more extensive weight losses. On *stock diet*, thyroids tended to be somewhat larger in moribund rats than in

Table 45.—*Thyroid weight in relation to body weight of rats maintaining weight on stock, SP 8 HVO, and SPE diets*

Diet and body weight (grams)	Rats	Average body weight	Thyroid (mg.) to body weight (grams)	Thyroid weight		Thyroids weighing—			
				Average	Range	Less than 10.0mg.	10.0 to 14.9mg.	15.0 to 19.9mg.	20.0 mg. and over
Stock:	*Number*	*Grams*	*Percent*	*Mg.*	*Mg.*	*Percent*	*Percent*	*Percent*	*Percent*
300 to 399	16	372	2. 5	9. 3	6. 0–13. 2	69	31	0	0
400 to 449	26	430	2. 5	10. 8	5. 6–21. 7	38	54	4	4
450 to 499	40	471	2. 4	11. 5	5. 8–17. 0	25	65	10	0
500 to 599	12	547	2. 4	13. 3	7. 5–18. 4	25	42	33	0
SP 8 HVO:									
400 to 499	10	466	2. 0	9. 4	6. 6–12. 0	60	40	0	0
500 to 599	20	547	2. 1	11. 4	6. 7–17. 5	35	50	15	0
600 to 699	26	646	1. 9	12. 5	8. 7–19. 9	19	69	12	0
700 and over	8	730	1. 9	13. 6	7. 8–17. 5	12	38	50	0
SPE:									
400 to 499	5	471	3. 0	14. 0	9. 1–16. 9	20	40	40	0
500 to 599	43	560	2. 8	15. 7	10. 3–25. 4	0	46	46	7
600 to 699	30	641	2. 5	16. 3	6. 1–26. 1	7	37	37	20
700 to 799	8	736	2. 4	18. 0	9. 5–21. 7	12	0	50	38

Table 46.—*Thyroid weights of rats losing weight at different ages on stock, SP 8 HVO, and SPE diets*

Weight loss, diet, and age (days)	Rats	Average age	Average weight loss	Thyroid weight		Thyroids weighing—				
				Average	Range	Less than 10.0 mg.	10.0 to 14.9 mg.	15.0 to 19.9 mg.	20.0 to 29.9 mg.	30.0 mg. and over
Losing less than 100 grams:										
Stock:	*Number*	*Days*	*Grams*	*Mg.*	*Mg.*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
300 to 699	5	592	56	11. 9	8. 6–17. 5	20	60	20	0	0
700 to 799	4	763	65	14. 7	9. 4–17. 7	25	25	50	0	0
800 and over	5	853	66	17. 1	14. 2–19. 9	0	40	60	0	0
SP 8 HVO:										
Less than 400	8	250	24	9. 5	5. 7–12. 5	50	50	0	0	0
400 to 599	12	491	61	13. 4	7. 2–19. 6	17	50	33	0	0
600 to 799	8	663	50	13. 0	5. 1–19. 4	12	50	38	0	0
SPE:										
Less than 300	9	185	23	11. 5	7. 5–14. 4	33	67	0	0	0
300 to 499	39	401	51	19. 8	11. 0–32. 9	0	15	46	31	8
500 to 699	17	590	52	24. 8	13. 3–36. 7	0	12	18	59	12
Losing more than 100 grams:										
Stock:										
300 to 699	8	521	125	14. 5	10. 8–18. 8	0	62	38	0	0
700 to 799	4	776	193	13. 7	12. 0–14. 8	0	100	0	0	0
800 and over	2	808	161	16. 1	14. 4, 17. 8	0	50	50	0	0
SP 8 HVO:										
Less than 500	5	422	155	20. 1	4. 4–30. 4	20	0	0	60	20
500 to 699	15	600	169	20. 4	9. 3–32. 4	7	13	40	20	20
700 and over	9	829	205	18. 9	8. 9–30. 8	11	22	33	11	22
SPE:										
Less than 400	20	352	150	22. 6	8. 3–37. 4	5	5	35	40	15
400 to 499	23	443	164	24. 2	7. 6–46. 1	4	0	26	48	22
500 to 699	32	583	160	25. 9	18. 8–36. 2	0	0	9	69	22

those maintaining weight, but the extent of the weight loss before sacrifice seemed to have no influence on the size of this gland. On *SP 8 HVO diet,* thyroids were similar in size to those observed in rats maintaining weight when the loss in body weight was less than 100 grams but were larger when the loss exceeded 100 grams. On *SPE diet,* a high proportion of the thyroids was large, even in rats that lost less than 100 grams. The thyroids of rats fed SPE diet were consistently larger than those from rats fed stock or SP 8 HVO diets for all groups as long as comparisons were confined to animals of similar age, taking into consideration extent of weight loss before sacrifice.

Thyroid weight and kidney damage.—In Table 47 are summarized data relating thyroid size to the histological findings in the kidneys from rats fed stock, SP 8 HVO, or SPE diets. Large thyroids were often associated with kidneys showing extensive damage. Even in rats that were maintaining weight, thyroid weight tended to parallel kidney damage. The extent and kind of damage in relation to the size of this organ, however, was influenced by diet and extent of weight loss. Calcium deposition in the kidneys, seen only in moribund rats, was frequently associated with enlarged thyroids. Eighty percent of the rats with thyroids exceeding 30 mg. had kidneys containing calcium deposits. Calcium was rarely found when thyroids weighed less than 20 mg. except in moribund SPE rats that had lost more than 100 grams before sacrifice. No data were available to establish how much of the weight recorded as thyroid was due to the parathyroid gland. Results from microscopic examination of

Table 47.—*Thyroid weight in relation to extent and kind of kidney damage for rats maintaining or losing weight on stock, SP 8 HVO, and SPE diets*

Weight status, diet, and thyroid weight (mg.)	Rats	Average thyroid weight	Histological ratings for kidneys			
			Hyalin	Cystic	Glomerular	Calcium
Rats maintaining weight:						
Stock:	*Number*	*Mg.*	*Score*	*Score*	*Score*	*Score*
Less than 13.0	65	10. 1	0. 2	0	0	0
13.0 to 14.9	10	14. 0	1. 2	. 5	. 7	0
15.0 to 19.9	9	16. 3	1. 6	. 8	. 8	0
SP 8 HVO:						
Less than 13.0	32	10. 0	. 2	0	0	0
13.0 to 14.9	2	14. 6	. 5	0	0	0
15.0 to 19.9	6	17. 3	1. 2	. 5	. 5	0
SPE:						
Less than 13.0	13	11. 1	. 8	. 2	. 2	0
13.0 to 14.9	16	14. 1	. 9	. 1	0	0
15.0 to 19.9	28	17. 0	1. 4	. 3	. 7	0
20.0 to 29.9	6	21. 6	1. 8	. 3	. 3	0
Rats losing less than 100 grams:						
Stock:						
Less than 13.0	5	10. 2	. 2	0	0	0
13.0 to 14.9	3	14. 6	1. 7	. 3	. 3	0
15.0 to 19.9	6	18. 1	2. 3	1. 5	2. 2	0
SP 8 HVO:						
Less than 13.0	16	9. 6	. 1	0	0	0
13.0 to 14.9	4	13. 8	1. 0	0	0	0
15.0 to 19.9	8	17. 7	1. 6	. 8	. 4	0
SPE:						
Less than 13.0	6	11. 4	1. 2	. 5	. 5	0
13.0 to 14.9	8	14. 2	1. 6	. 4	. 2	0
15.0 to 19.9	21	17. 2	1. 7	1. 0	. 6	. 1
20.0 to 29.9	22	25. 3	2. 1	2. 2	1. 6	. 8
30. 0 and over	5	33. 7	2. 6	3. 6	2. 0	0
Rats losing more than 100 grams:						
Stock:						
Less than 13.0	3	11. 7	2. 3	1. 7	1. 7	0
13.0 to 14.9	6	14. 3	2. 7	1. 7	2. 3	0
15.0 to 19.9	4	17. 1	2. 0	2. 8	2. 8	. 5
SP 8 HVO:						
Less than 15.0	7	9. 7	1. 1	. 3	. 3	. 3
15.0 to 19.9	9	17. 2	2. 2	1. 3	1. 1	. 7
20.0 to 29.9	7	23. 5	2. 4	2. 3	1. 6	1. 0
30.0 and over	6	31. 6	3. 2	2. 8	2. 8	1. 8
SPE:						
Less than 15.0	3	9. 9	2. 7	2. 3	1. 3	2. 0
15.0 to 19.9	16	17. 8	2. 6	3. 2	2. 0	1. 4
20.0 to 29.9	41	24. 5	2. 5	3. 2	2. 1	1. 6
30.0 and over	14	34. 6	2. 4	3. 1	2. 1	2. 3

these glands have indicated that the large thyroids from these moribund rats were reflecting, in part at least, enlarged parathyroid glands. More details (*54*) are reported in a separate communication dealing with the thyroid and parathyroid glands from these rats as seen from microscopic examination.

Rats fed SPM, SPB, and SPPB diets.—The thyroid weights of rats fed SPM, SPB, and SPPB diets are summarized in table 48. Thyroids similar in size to those found in rats fed stock or SP 8 HVO diets were obtained from rats maintaining weight on SPM diet; those from rats 300 to 600 days of age fed SPB or SPPB diets tended to be somewhat larger. Enlarged thyroids were seen frequently with SPPB diet, even in rats less than 500 days of age regardless of weight loss. Thyroids exceeding 20 mg. in weight were found occasionally in animals that had lost more than 100 grams on each of the three diets, as well as in older rats with weight loss less than 100 grams. The thyroids from rats with a relatively long life span on SPM diet tended to be small in spite of the large weight loss before sacrifice.

Rats fed other experimental diets.—In table 49 are summarized data for the thyroid weights of rats fed the remaining experimental diets; the results are for rats that were losing weight, with the few exceptions indicated in this table. Interpretation of the results in terms of dietary response is complicated by variation in weight loss in the different experimental series. Data on rats maintaining weight or with weight loss limited before sacrifice might reveal differences due to diet that are not apparent from the data available.

Thyroids were consistently large, regardless of weight loss, for rats fed the various SPE supplemented diets, and were similar in size to thyroids obtained from rats fed the unsupplemented SPE diet. The kind of fat (HVO, lard, or butter) or the level of fat (8 or 16 percent) appeared to be without influence on the size of this gland. Replacement of egg white for casein in the semipuri-

Table 48.—*Thyroid weights for rats maintaining or losing weight at different ages on SPM, SPB, and SPPB diets*

Weight status, diet, and age of rats (days)	Rats	Average age	Average body weight	Average weight loss	Thyroid weights		Thyroids weighing—			
					Average	Range	Less than 10.0 mg.	10.0 to 14.9 mg.	15.0 to 19.9 mg.	20.0 mg. and over
Rats maintaining weight:										
SPM:	*Number*	*Days*	*Grams*	*Grams*	*Mg.*	*Mg.*	*Percent*	*Percent*	*Percent*	*Percent*
Less than 300	13	214	553	0	10. 5	4. 3–14. 7	46	54	0	0
300 to 499	6	399	672	0	11. 2	4. 2–16. 7	33	50	17	0
500 to 599	2	550	624	0	11. 6	7. 8, 15. 5	50	50	0	0
SPB:										
Less than 300	13	214	520	0	11. 5	8. 1–15. 1	38	54	8	0
300 to 499	7	413	596	0	13. 1	7. 0–27. 1	29	57	0	14
500 to 599	3	549	673	0	16. 1	14. 1–17. 5	0	33	67	0
SPPB:										
Less than 300	14	217	535	0	12. 5	8. 2–19. 0	21	57	21	0
300 to 499	5	412	644	0	16. 0	13. 3–19. 8	0	60	40	0
500 to 599	6	550	728	0	15. 8	9. 6–22. 0	17	33	17	33
Rats losing less than 100 grams:										
SPM:										
Less than 500	11	358	591	49	13. 6	8. 4–18. 2	18	45	36	0
500 and over	6	599	748	44	19. 1	11. 9–34. 7	0	50	17	33
SPB:										
Less than 500	9	308	508	46	12. 8	6. 2–19. 9	33	33	33	0
500 and over	7	671	628	53	15. 0	5. 7–23. 8	14	29	43	14
SPPB:										
Less than 500	22	338	576	42	17. 2	6. 2–27. 6	5	32	32	32
500 and over	10	625	618	66	16. 4	12. 2–20. 5	0	40	40	20
Rats losing more than 100 grams:										
SPM:										
Less than 500	6	421	532	144	22. 2	7. 1–46. 5	17	17	17	50
500 and over	8	776	563	198	15. 7	4. 6–30. 1	12	38	25	25
SPB:										
Less than 500	5	450	469	186	22. 1	12. 3–28. 4	0	20	20	60
500 and over	13	616	505	186	23. 1	10. 8–39. 0	0	8	31	62
SPPB:										
Less than 500	5	397	550	192	18. 8	9. 5–29. 0	20	20	20	40
500 and over	7	570	522	177	24. 4	13. 3–36. 5	0	14	14	71

TABLE 49.—*Thyroid weights of rats fed other diets*

Strain and diet	Rats	Average age	Average weight loss	Thyroid weights	
				Average	Range
BHE rats					
SPE supplemented with—	*Number*	*Days*	*Grams*	*Mg.*	*Mg.*
Choline, 0.5%	22	474	136	25. 4	12. 9–42. 5
B_{12}, 0.01 mg./100 gm	8	463	131	23. 4	17. 3–33. 5
Choline, 0.5%+B_{12}, 0.01 mg./100 gm	9	437	96	24. 1	18. 2–32. 8
B_6, 0.5 mg./100 gm	7	465	180	23. 8	18. 0–35. 7
Choline, 0.5%+B_6, 0.5 mg./100 gm	9	412	218	21. 0	9. 7–29. 7
Choline, 0.5%+B_{12}, 0.01 mg./100 gm.+B_6, 0.5 mg./100 gm	10	430	138	24. 2	13. 5–39. 8
Cholesterol, 0.46%	7	460	155	22. 2	19. 0–29. 8
Cholesterol, 1.38%	9	406	142	23. 3	16. 7–29. 4
Ascorbic acid, 0.2%	8	436	129	23. 2	18. 1–28. 6
Ascorbic acid, 0.2%+cholesterol, 0.46%	9	434	183	25. 4	13. 6–36. 0
SP 16 HVO	4	586	77	14. 2	12. 0–15. 7
SP 8 lard	15	553	98	19. 9	13. 7–30. 1
SP 16 lard	7	514	109	18. 0	11. 2–25. 2
SP 8 butter	5	528	80	17. 9	10. 6–27. 8
SP 16 butter	10	503	66	15. 4	10. 0–23. 3
SPa 16 HVO	17	637	172	19. 0	8. 7–32. 6
SPb 8 HVO	9	632	118	16. 8	7. 3–26. 9
SPE (fresh egg)	6	483	166	23. 7	7. 6–36. 0
SPEW	6	530	155	19. 4	9. 2–28. 2
SPEY	7	473	178	24. 1	18. 1–31. 4
SPW 8 HVO	6	550	0	10. 3	7. 2–13. 9
E100	21	541	129	19. 5	9. 0–45. 8
Y100	19	400	73	17. 9	9. 5–28. 8
Littermates fed—					
SPE	5	428	66	15. 6	13. 8–17. 6
Y100	5	425	35	19. 7	13. 1–28. 8
Y97+salt mixture, 3.0%	5	430	5	16. 6	12. 9–19. 5
Diet reversal:					
Sacrificed at approximately 250 days:					
Stock	3	250	0	12. 4	11. 9–12. 7
SPE	3	249	0	16. 9	14. 5–20. 8
Continued on—					
Stock	2	574	207	21. 5	14. 6, 28. 4
SPE	2	396	133	17. 2	17. 1, 17. 4
Reversed at 250 days:					
Stock changed to SPE	4	686	109	19. 7	13. 0–22. 8
SPE changed to stock	3	577	128	22. 4	21. 3–23. 6
Wistar rats					
SP 8 HVO	9	727	106	16. 1	11. 3–30. 9
SPE	9	664	80	22. 2	15. 3–34. 1
BHE rats of parents fed Wistar stock diet					
SP 8 HVO	9	703	74	12. 9	5. 8–20. 6
SPE	9	456	81	21. 7	9. 9–49. 6

fied diet resulted in thyroids similar in size to those from SP 8 HVO rats. Although large thyroids were occasionally found in rats fed the diet containing 100 percent egg, the average of 18.0 mg. for the thyroids from 16 rats losing more than 100 grams on this diet was considerably less than the 24-mg. average observed for comparable SPE rats. On the basis of the limited data available, there was no evidence that supplementation of the diet consisting of 100 percent egg yolk with salt mixture had any marked influence on thyroid weight. When feeding of SPE diet was initiated at 250 days, thyroids tended to be smaller than when this diet was fed throughout life, but more data are needed to establish the significance of this difference. The thyroids from Wistar rats fed SP 8 HVO diet and SPE diet were similar in size to those from comparable BHE rats fed these diets, and no evidence for strain differences was apparent from data on moribund rats.

DISCUSSION.—Donaldson (*50*) reported a value of 60.9 mg. for the total thyroid weight in male rats weighing 450 grams. A linear relation between body and thyroid weight was observed.

Freudenberger (*70*) reported still larger thyroid weights of 83 mg. for the Long-Evans strain of rats, and observed significant differences between the Wistar and Long-Evans strains whether considered in terms of absolute value or in relation to body size. The thyroid weights reported by these investigators are considerably larger than those reported in this publication for BHE or Wistar rats. The lack of the close correlation between this gland and body weight such as has been reported by Donaldson (*50*) may be due to the large weights of many of the animals, which undoubtedly represent, in part at least, fat deposition rather than growth of active tissues.

Enlargement of the thyroid gland may be associated with a low iodine intake. Low iodine intake, however, did not appear to be responsible for the enlarged glands observed with SPE diet which contained 1 p.p.m. iodine. Thompson (*182*) reported evidence that the occurrence of hyperplasia in the thyroid gland may be influenced by the relative amount of iodine and calcium in the diet. Differences in the relative amounts of these two elements also fail to explain the differences observed with diet in the size of the thyroids of BHE rats. The possibility of some other mineral imbalance in the diets investigated has not been excluded.

Summary.—Diet appeared to influence the size of the thyroid more than that of the adrenal gland in rats that were *maintaining weight* when sacrificed. The largest thyroids were found in rats fed SPE diet; on SPPB diet this gland also tended to be large. The influence of age on thyroid size varied with the diet. Thyroid size tended to parallel body weight but, as with the adrenals, the correlation coefficients were relatively low.

Thyroid size varied with age and extent of weight loss as well as with diet in rats that were *losing weight* when sacrificed. Large thyroids were often associated with kidneys showing extensive damage. Although with some diets, calcium deposition tended to parallel thyroid size, possibly owing to parathyroid enlargement, calcium deposits in the kidneys of rats fed SPPB diet occurred rarely in spite of thyroid enlargement.

The thyroid weights for moribund Wistar rats fed SP 8 HVO or SPE diet were similar to those for comparable BHE rats, and provide no evidence for strain differences with regard to thyroid size.

Thymus weight

Data on the thymus weights of BHE rats were obtained for a limited number of diets, and the results are summarized in table 50. A marked decrease occurred in the weight of this gland between 150 and 250 days of age regardless of the diet, followed by a relatively small decrease with age thereafter. Donaldson (*50*) reported heavier thymus glands for Wistar rats of comparable age, with glands weighing 211 mg. at 150 days of age and 123 mg. at 250 days.

Table 50.—*Thymus weights of rats at different ages on stock, SP 8 HVO, SPE, SPM, SPB, and SPPB diets*

Diet and age of rats (days)	Rats	Average age	Thymus weight	
			Average	Range
	Number	*Days*	*Mg.*	*Mg.*
Stock:				
Less than 200	15	148	106	52–155
200 to 299	11	258	66	36–138
300 to 499	8	454	44	28– 58
500 and over	19	586	33	20– 48
SP 8 HVO:				
Less than 200	5	154	151	100–196
200 to 299	7	254	65	36– 80
300 to 499	4	417	69	41– 86
500 and over	10	650	48	28– 72
SPE:				
Less than 200	6	140	132	92–178
200 to 299	14	270	71	49–101
300 to 499	13	337	57	22–113
500 and over	11	510	33	22– 45
SPM:				
Less than 200	5	154	118	64–167
200 to 299	5	249	58	46– 70
300 to 499	5	441	49	40– 72
500 and over	4	793	44	25– 64
SPB:				
Less than 200	5	154	125	81–164
200 to 299	5	250	57	38– 82
300 to 499	6	426	45	27– 83
500 and over	7	633	44	31– 56
SPPB:				
Less than 200	5	155	152	78–193
200 to 299	5	250	75	55– 92
300 to 499	2	340	50	33, 66
500 and over	9	634	63	45– 97

Chemical Investigations

Kidney

Stock, SP 8 HVO, and SPE diets.—Data for moisture, protein, fat, and ash in the kidneys from rats fed stock, SP 8 HVO, and SPE diets are summarized in table 51. The results for percentage composition on a dry-weight basis and for total content are included.

The data for rats maintaining weight were from fasted animals except for the two age groups indicated for stock rats. The results for young fasted rats fed stock diet were similar to those for older nonfasted rats. Neither diet nor age was found to influence the composition of the kidneys from these animals. Differences in content were related chiefly to the size of the kidney.

Separation of the data for moribund rats was on the basis of weight loss only. The results for nonfasted and fasted moribund rats have been combined because there was no apparent difference in the composition of the kidneys from these two groups of animals. The kidneys from rats that had lost less than 100 grams were similar in composition to the kidneys from rats that were maintaining their weight at the time of sacrifice. The large kidneys from rats that lost more than 100 grams tended to have a high percentage of moisture and protein and a low percentage of fat.

TABLE 51.—*Protein, fat, and ash in kidneys from rats maintaining or losing weight at different ages on stock, SP 8 HVO, and SPE diets*

Weight status, diet, and age of rats (days)	Rats	Average age	Average kidney weight	Water	Based on dry weight						Total content		
					Protein		Fat		Ash		Protein	Fat	Ash
					Average	Range	Average	Range	Average	Range			
Maintaining weight:	*Number*	*Days*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Mg.*	*Mg.*	*Mg.*
Stock:													
Less than 300	9	224	1. 42	75. 3	78. 5	75. 8–80. 2	13. 3	13. 0–13. 6	4. 5	3. 5–5. 1	276	47	16
300 to 499 [1]	5	412	1. 74	75. 8	80. 1	79. 1–81. 6	14. 9	13. 6–15. 8	5. 1	5. 0–5. 2	369	63	22
500 to 699 [1]	5	550	2. 28	78. 5	78. 9	78. 7–79. 2	11. 6	10. 7–12. 1	5. 3	5. 2–5. 4	386	56	26
SP 8 HVO:													
Less than 300	6	252	1. 41	75. 5	78. 2	74. 8–81. 7	14. 9	12. 8–17. 8	5. 1	4. 1–5. 6	271	51	18
300 to 499	15	402	1. 60	76. 4	78. 7	74. 7–82. 6	14. 2	12. 1–17. 0	4. 7	3. 5–6. 6	296	53	18
500 to 599	11	547	2. 23	76. 7	79. 4	76. 4–81. 4	14. 0	10. 1–15. 8	5. 0	3. 3–6. 4	398	68	26
SPE:													
Less than 300	7	252	1. 68	77. 2	80. 4	76. 0–84. 1	14. 2	13. 1–15. 8	5. 2	3. 5–6. 5	309	54	20
300 to 399	7	353	2. 41	78. 2	79. 3	74. 5–86. 1	13. 3	12. 7–14. 2	5. 2	4. 4–5. 6	407	68	27
400 to 599	14	481	2. 06	77. 6	79. 2	76. 6–82. 1	12. 9	9. 5–16. 4	4. 6	3. 1–6. 3	361	58	20
Losing less than 100 grams:													
Stock	5	829	2. 34	80. 1	82. 5	81. 9–82. 9	12. 3	11. 1–13. 1	5. 7	5. 2–6. 5	377	56	27
SP 8 HVO	12	460	1. 94	77. 0	80. 8	74. 7–86. 6	14. 6	11. 2–18. 1	4. 2	2. 9–5. 7	329	60	17
SPE	20	356	3. 98	79. 1	81. 7	76. 6–89. 0	11. 7	5. 5–16. 2	5. 0	1. 8–6. 5	606	70	37
Losing more than 100 grams:													
Stock	4	626	3. 74	81. 2	87. 5	86. 4–90. 0	8. 0	7. 2– 9. 7	4. 8	4. 3–5. 6	613	56	35
SP 8 HVO	15	711	4. 87	82. 1	84. 4	77. 2–93. 0	10. 2	2. 6–14. 5	5. 3	2. 7–8. 0	718	78	43
SPE	16	398	7. 56	82. 1	85. 8	79. 4–90. 7	9. 0	6. 5–14. 3	6. 1	3. 7–9. 7	1160	116	80

[1] Nonfasted rats.

Table 52, in which the results are separated on the basis of kidney weight, shows more clearly this relationship between kidney size and composition. The results for kidneys of the same size were similar regardless of age, diet, or weight loss. The percentage of fat in the kidney tended to decrease and the protein to increase with kidney weight. Although total content of both protein and fat tended to increase with kidney size, enlargement of this organ was due in large part to increased protein. Ash content also increased consistently with increasing kidney weight, but no consistent relation between the percentage of ash and kidney size was observed. High ash values were found most frequently in rats fed SPE diet, but values exceeding 6 percent were obtained occasionally in stock and SP 8 HVO rats. Calcium deposition in the kidney did not necessarily parallel the percentage of ash in this organ. Determination of ash in the kidney was the least accurate of the measurements made, because of the relatively small samples available for analysis.

SPM, SPB, AND SPPB DIETS.—In table 53 are summarized data for the composition of kidneys from rats fed SPM, SPB, and SPPB diets. With these diets, there was little evidence that age or diet influenced the composition of the kidneys from rats that were maintaining their weight, except for the low ash in the kidneys of young SPPB rats. The higher content of fat, protein, and ash in rats 400 to 600 days old was a reflection of the larger kidneys in these older animals. In the moribund rats with weight loss over 100 grams, the trend for a high percentage of protein and a low percentage of fat in the large kidneys of SPB and SPPB rats was again apparent. The percentage of ash rarely exceeded 6.0 percent and values for rats fed SPB and SPPB diets were similar, although calcium deposits were seen more frequently in kidneys from SPB rats.

OTHER EXPERIMENTAL DIETS.—In table 54 are summarized protein, fat, and ash data for the kidneys from rats on additional experimental diets. Analyses were not made for kidneys from rats on all of the diets under investigation. Again, the differences observed tended to parallel kidney size. The low percentages of fat in kidneys from rats fed supplemented SPE diets were similar to those found on the unsupplemented diet, and were characteristic of those observed with large kidneys regardless of diet. The tendency for ash values to be high in the kidneys from rats fed the supplemented SPE diets was comparable to that observed in moribund rats on the unsupplemented diet.

WISTAR RATS FED SP 8 HVO AND SPE DIETS.—Kidneys from Wistar rats fed SP 8 HVO diet were similar in composition to those of the same size from BHE rats. Kidneys from Wistar rats fed SPE diet differed from those in moribund BHE rats fed this diet, as might be expected considering the differences in kidney size. The relatively high percentage of fat in the kidneys of Wistar rats fed SPE diet was of questionable significance in view of the limited data available. Ash values were generally low when compared with those found in the kidneys of BHE rats fed SP 8 HVO or SPE diets.

SUMMARY.—The influence of diet on the composition of the kidney depended chiefly on its influence on the size of this organ. Enlargement of the kidney was accompanied by an increase in percentage of protein on the dry-weight basis as well as in total protein content, and by a decrease in the percentage of fat.

High ash values were found most frequently in rats fed SPE diet. Calcium deposition in the

TABLE 52.—*Protein, fat, and ash in kidneys of different weights from rats fed stock, SP 8 HVO, and SPE diets*

Diet and range of kidney weights (grams)	Rats	Average kidney weight	Water	Based on dry weight			Total content		
				Protein	Fat	Ash	Protein	Fat	Ash
	Number	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Mg.*	*Mg.*	*Mg.*
Stock:									
Kidney less than 2.00	20	1.65	76.4	79.6	13.5	4.9	316	52	19
2.00 to 2.99	7	2.74	78.9	81.7	11.7	5.7	465	67	33
3.00 to 4.99	4	3.74	81.2	87.5	8.0	4.8	613	56	34
SP 8 HVO:									
Kidney less than 2.00	40	1.64	76.5	79.2	14.6	4.7	304	56	18
2.00 to 2.99	5	2.37	76.8	80.2	13.7	5.2	442	75	29
3.00 to 4.99	8	4.19	82.6	85.5	9.4	5.6	627	64	41
5.00 to 9.99	6	6.94	83.3	84.4	9.1	4.5	979	103	53
SPE:									
Kidney less than 2.00	30	1.59	76.7	80.1	14.0	5.0	297	51	19
2.00 to 2.99	10	2.40	80.9	79.3	12.6	4.0	400	64	21
3.00 to 4.99	7	3.73	80.1	82.1	11.1	4.6	606	82	34
5.00 to 9.99	19	6.90	82.5	85.2	8.5	6.1	1027	102	72
10.0 and over	6	11.1	82.7	87.0	8.1	5.3	1675	153	96

TABLE 53.—*Protein, fat, and ash in kidneys from rats maintaining or losing weight on SPM, SPB, and SPPB diets*

Weight status, diet, and age of rats (days)	Rats	Average age	Average kidney weight	Water	Based on dry weight						Total content		
					Protein		Fat		Ash				
					Average	Range	Average	Range	Average	Range	Protein	Fat	Ash
	Number	*Days*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Mg.*	*Mg.*	*Mg.*
Maintaining weight:													
SPM:													
200 to 399	6	304	1. 37	76. 1	80. 1	78. 4–81. 2	13. 5	10. 1–15. 8	4. 0	2. 6–6. 1	263	43	13
400 to 599	7	498	1. 87	77. 4	80. 7	77. 4–85. 3	13. 0	10. 3–14. 9	4. 1	3. 4–5. 5	331	53	17
SPB:													
200 to 399	6	305	1. 52	76. 8	80. 0	78. 9–81. 2	14. 3	11. 8–17. 2	4. 3	3. 2–6. 9	280	50	15
400 to 599	6	498	2. 32	77. 8	79. 8	78. 1–84. 1	15. 3	10. 0–20. 2	4. 2	3. 8–5. 2	380	68	21
SPPB:													
200 to 399	5	295	1. 49	77. 2	80. 4	78. 0–81. 7	13. 9	11. 9–16. 0	2. 5	2. 0–3. 2	272	48	8
400 to 599	12	523	1. 98	77. 5	80. 5	77. 0–82. 8	14. 5	11. 8–18. 1	4. 3	2. 7–6. 3	350	63	19
Losing less than 100 grams:													
SPM	10	485	2. 75	78. 8	77. 8	71. 0–87. 2	13. 0	8. 0–14. 7	4. 4	3. 4–4. 9	444	70	25
SPB	10	421	2. 16	77. 3	77. 2	75. 3–80. 7	10. 2	7. 0–14. 9	4. 4	4. 0–5. 8	369	48	22
SPPB	10	415	3. 90	77. 6	76. 6	71. 9–82. 3	13. 9	9. 2–23. 1	4. 5	4. 2–5. 1	506	89	33
Losing more than 100 grams:													
SPM	5	635	2. 69	82. 9	80. 3	78. 1–86. 1	14. 6	9. 1–17. 2	4. 5	3. 4–5. 3	355	59	19
SPB	3	639	5. 80	85. 2	84. 2	82. 6–87. 5	6. 6	3. 5–12. 8	4. 2	3. 8–4. 4	726	62	35
SPPB	4	561	5. 10	82. 9	82. 1	76. 1–85. 4	10. 7	9. 9–11. 9	5. 1	3. 1–6. 4	699	92	42

TABLE 54.—*Protein, fat, and ash in kidneys from rats fed other experimental diets*

Strain and diet	Rats	Average age	Average weight loss	Average kidney weight	Water	Based on dry weight: Protein	Based on dry weight: Fat	Based on dry weight: Ash	Total content: Protein	Total content: Fat	Total content: Ash
BHE rats	*Number*	*Days*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Mg.*	*Mg.*	*Mg.*
SPE supplemented with—											
Choline, 0.5%	19	492	139	6. 33	82. 1	85. 4	9. 0	5. 9	950	95	67
B_{12}, 0.01 mg./100 gm	8	463	131	7. 11	81. 8	78. 8	9. 6	5. 2	954	105	69
Choline, 0.5%+B_{12}, 0.01 mg./100 gm	10	434	114	7. 18	82. 3	82. 5	10. 3	5. 8	963	112	71
B_6, 0.5 mg./100 gm	7	502	129	5. 13	79. 5	82. 0	9. 3	5. 6	824	89	58
Choline, 0.5%+B_6, 0.5mg./100gm	9	412	127	4. 74	80. 5	82. 8	10. 2	5. 4	724	80	49
Choline, 0.5%+B_{12}, 0.01 mg./100 gm.+B_6, 0.5 mg./100 gm	10	431	129	5. 76	81. 4	80. 8	10. 3	6. 6	896	110	72
Cholesterol, 0.46%	8	451	150	7. 54	81. 5	83. 2	11. 0	5. 1	1, 240	155	76
Cholesterol, 1.38%	10	409	142	6. 83	81. 6	85. 3	10. 1	6. 8	971	109	78
Ascorbic acid, 0.2%	9	415	115	5. 00	80. 1	83. 0	8. 6	5. 2	802	76	50
Ascorbic acid, 0.2%+cholesterol, 0.46%	9	434	183	5. 74	80. 2	85. 1	9. 9	6. 0	940	109	67
SP 16 HVO	8	629	84	2. 76	77. 9	78. 9	13. 4	4. 7	446	71	26
SP 8 lard	11	557	99	3. 26	79. 5	82. 2	11. 6	4. 2	540	71	26
SP 16 lard	9	572	117	3. 31	79. 7	82. 2	13. 2	4. 7	529	81	30
SP 8 butter	8	601	82	2. 58	79. 3	80. 5	12. 4	4. 0	400	59	20
SP 16 butter	9	486	72	2. 74	78. 0	79. 3	13. 5	4. 8	459	69	29
E100	9	559	141	4. 36	82. 9	82. 7	13. 6	5. 6	523	86	35
Y100	14	375	80	2. 42	79. 4	79. 5	13. 7	6. 2	363	61	31
Littermates fed—											
SPE	5	428	66	3. 88	79. 5	83. 5	9. 9	6. 5	637	70	54
Y100	4	420	42	2. 54	80. 2	79. 4	14. 6	6. 0	383	69	29
Y97+salt mixture, 3.0%	4	424	6	1. 66	77. 8	80. 8	13. 5	5. 7	299	50	21
Diet reversal:											
Sacrificed at approx. 250 days:											
Stock	3	250	0	2. 07	78. 8	79. 8	13. 2	3. 0	341	56	13
SPE	3	249	0	2. 74	81. 3	76. 8	12. 8	2. 0	375	61	10
Continued on—											
Stock	3	590	195	4. 25	81. 3	84. 7	8. 1	------	654	63	------
SPE	2	396	133	4. 64	84. 5	78. 8	9. 3	5. 9	565	67	41
Reversed at 250 days:											
Stock changed to SPE	4	686	109	3. 94	80. 9	84. 2	9. 2	4. 9	624	67	35
SPE changed to stock	3	577	116	3. 55	80. 6	82. 4	9. 5	3. 8	560	63	26
Wistar rats											
SP 8 HVO	8	762	119	2. 02	80. 0	82. 0	13. 5	4. 3	323	54	18
SPE	5	772	103	2. 80	79. 6	80. 9	16. 4	4. 3	446	89	23

kidney and a high percentage of ash did not necessarily parallel one another.

Differences between the composition of kidneys from BHE and Wistar rats were due chiefly to differences in the kidney weights of these two strains of animals.

Urinary protein

BHE RATS.—Data on urinary protein excretion were obtained for BHE rats fed 11 of the diets under investigation, and the results are summarized in table 55. Urine was collected under two conditions: (1) a 7-hour collection period without access to food; (2) a 17-hour collection period with access to food. The conditions of collection are indicated in the table, and the urinary protein values recorded are for total protein excreted during the collection period. Results are grouped by age at which urine collection was made; in most cases the age range within groups was less than 2 weeks. Data for age at death and size of kidney are included to relate as well as possible the protein excretion with age at death, although urine samples were not collected at this time.

The amount of protein excreted by rats under 200 days of age was generally small regardless of diet. When urine collections were made for a 7-hour period without access to food, protein excretion was less than 10 mg. for 67 percent of the rats. Only 3 of the 61 rats in this group excreted more than 50 mg. of protein.

When data obtained under comparable conditions were available, a tendency for increased protein excretion with increasing age was apparent. Dietary differences were observed in the older

TABLE 55.—*Urinary protein of fasted and nonfasted rats at different ages on various diets* [1]

Strain, diet, and conditions for urine collection	Rats	Average age	Total urine protein		Protein excretion of—											
			Average	Range	Less than 10 mg.			10 to 49 mg.			50 to 99 mg.			100 mg. and over		
					Rats	Age at death	Kidney weight	Rats	Age at death	Kidney weight	Rats	Age at death	Kidney weight	Rats	Age at death	Kidney weight
BHE rats																
	Number	*Days*	*Mg.*	*Mg.*	*Number*	*Days*	*Grams*	*Number*	*Days*	*Grams*	*Number*	*Days*	*Grams*	*Number*	*Days*	*Grams*
Stock:																
Fasted	12	372	17	2–73	6	----	----	5	----	----	1	----	----	0	----	----
	5	471	56	0–96	2	----	----	0	----	----	3	----	----	0	----	----
SP 8 HVO:																
Fasted	10	417	19	1–83	7	724	3.62	2	673	5.58	1	422	1.92	0	----	----
	2	826	160	99, 220	0	----	----	0	----	----	1	889	3.31	1	846	3.40
Nonfasted	10	180	9	4–21	7	740	3.91	3	552	3.78	0	----	----	0	----	----
	13	391	44	2–244	3	646	3.27	7	573	3.75	2	703	2.89	1	587	4.91
SPE:																
Fasted	21	114	10	2–27	13	309	2.45	8	298	3.64	0	----	----	0	----	----
	6	366	87	6–141	1	580	8.84	1	629	3.22	1	423	11.63	3	443	6.85
Nonfasted	9	375	126	2–473	2	609	7.39	2	530	6.23	3	490	7.09	2	414	11.89
SPM:																
Nonfasted	13	374	29	2–213	7	674	3.32	5	610	2.98	0	----	----	1	464	4.8
SPB:																
Nonfasted	14	394	42	4–161	3	725	3.55	6	538	5.14	4	510	5.42	1	450	4.51
SPPB:																
Nonfasted	11	395	62	4–310	2	621	2.20	6	624	3.32	0	----	----	3	552	5.67
SPa:																
Fasted	10	417	17	1–85	7	771	3.97	2	590	3.91	1	542	7.92	0	----	----
Nonfasted	10	180	17	4–53	5	808	3.95	4	617	4.46	1	613	5.98	0	----	----
SPE (fresh egg):																
Fasted	10	155	15	2–55	6	512	6.59	3	443	10.54	1	421	8.32	0	----	----
SPEW:																
Fasted	10	155	6	1–15	8	621	3.97	2	407	9.60	0	----	----	0	----	----
SPEY:																
Fasted	10	155	24	0–95	6	512	5.58	2	412	6.04	2	500	5.58	0	----	----
E100:																
Fasted	10	155	6	2–11	8	555	5.57	2	520	3.22	0	----	----	0	----	----
	9	416	56	2–151	1	669	1.52	4	598	3.35	3	565	7.00	1	460	3.06
Nonfasted	10	180	24	6–125	4	613	4.50	5	553	4.19	0	----	----	1	358	4.98
Diet reversal:																
Rats continued on—																
Stock:																
Fasted	[2] 3	315	104	24–228	0	----	----	1	623	2.76	1	727	4.96	1	420	5.04
	3	412	140	88–238	0	----	----	0	----	----	2	675	3.86	1	420	5.04
SPE:																
Fasted	[2] 3	315	92	64–113	0	----	----	0	----	----	1	629	3.22	2	396	4.64
	1	411	59	59	0	----	----	0	----	----	1	629	3.22	0	----	----
Reversed at 250 days:																
Stock changed to SPE:																
Fasted	[2] 4	315	27	5–71	2	755	3.45	1	701	4.18	1	532	4.70	0	----	----
	4	412	50	30–94	0	----	----	3	737	3.69	1	532	4.70	0	----	----
SPE changed to stock:																
Fasted	[2] 4	315	68	17–217	0	----	----	3	734	4.04	0	----	----	1	384	4.38
	3	411	45	34–54	0	----	----	2	747	4.11	1	707	3.89	0	----	----
Wistar rats																
SP 8 HVO:																
Fasted	6	664	11	0–39	4	854	1.92	2	848	3.08	0	----	----	0	----	----
SPE:																
Fasted	6	664	9	0–20	4	846	2.18	2	794	2.52	0	----	----	0	----	----

[1] Urine collected for 7 hours from fasted rats; for 17 hours from nonfasted rats.
[2] Data for the two age groups on each diet were for the same rat if still alive.

rats. Of the nonfasting rats approximately 375 days old, those fed SPE diet excreted the most protein. Differences in excretion by rats fed SP 8 HVO, SPM, or SPB diets were small. Rats fed SPPB diet tended to excrete somewhat more protein, and the excretion of 3 of the 11 rats exceeded 100 mg.

Wistar rats.—Protein excretion by Wistar rats was low on SP 8 HVO and SPE diets, in marked contrast to the results with BHE rats. For a 7-hour collection period, urinary protein averaged 9 mg. for fasted 614-day-old Wistar rats fed SPE diet. No data were available for a comparable age group of BHE rats because of their short survival on this diet. However, by approximately 350 days, BHE rats were excreting 87 mg. of protein when urine was collected under comparable conditions.

Urinary protein and kidney damage.—Most measurements of urinary protein were made considerably before the age at which the animals died, and provide little direct evidence to relate extent of protein excretion to kidney damage or age of survival. The low urinary protein from rats less than 200 days old gave little indication of the extent of kidney damage at death or of the lifespan of the animals. The only young rat (180 days) to excrete over 100 mg. of protein had a 5-gram kidney when he died at the relatively early age of 358 days. In rats 350 to 400 days of age, urinary protein appeared to be a fairly good index of kidney damage and expected survival, with lifespan generally decreasing as urinary protein increased.

Discussion.—The results for BHE and Wistar rats reported in this publication confirm the findings of other investigators that some protein may be excreted by animals with kidneys that appear normal in all respects. Urinary protein for BHE rats was considerably greater than that generally reported by other investigators. Gilson (*72*) found proteinuria to be a usual occurrence in Wistar and Sprague-Dawley-Holtzman strains of rats. The average excretion during fasting was 3.0 mg. globulin and 3.3 mg. albumin in 24 hours. Particularly heavy protein precipitates were observed in the urine of a group of animals maintained at a temperature of 4°C. for 3 months. Saxton and Kimball (*168*) reported appreciable excretion of albumin by rats over 300 days old. Proteinuria was found to increase with age, although there was somewhat reduced frequency of protein excretion in rats over 800 days old. McCay, Maynard, Sperling, and Osgood (*121*) obtained an average daily protein excretion of 23 mg. for rats on a low level of dietary protein and 82 mg. for those on a high level. Albumin was found in the urine of mature rats with normal kidneys, but with chronic nephrosis increased amounts were present. There appeared to be a rough correlation between kidney damage and increased protein excretion, although the lifespan of the animals did not appear to be related to the latter. Rather (*155*) suggests that the threshold of the kidney to protein may be due to tubular resorption with proteinuria occurring if tubular resorption capacity is exceeded. Using rabbit anti-rat kidney serum to produce nephrotic rats, Drabkin and Marsh (*51*) observed a marked increase in labeled urinary protein after injection of labeled glycine into the nephrotic animals. Total serum protein decreased and the albumin moiety almost completely disappeared.

Summary.—Urinary protein excretion in BHE rats tended to increase with age and to be influenced by diet. The extent of the proteinuria observed appeared to parallel the occurrence of degenerative changes in the kidneys of these animals. Protein excretion in the urine was considerably greater than has generally been observed and seemed to be related to the shorter lifespan of these rats.

Protein excretion by Wistar rats was generally small and within the range reported by other investigators.

Liver

Rats maintaining weight on stock, SP 8 HVO, and SPE diets.—In table 56 are summarized data for protein, fat, and ash in livers of rats maintaining weight on stock, SP 8 HVO, or SPE diets, and included are results for percentage composition and total content. Most of the results recorded are for fasted rats. Limited data were obtained for nonfasted stock rats 300 days of age and older and for a group of young rats less than 300 days old fed SPE diet.

Age appeared to have little influence on the composition of livers from rats fed stock diet when the glycogen content of the livers from nonfasted rats was taken into consideration. Values of 76.2 percent for protein, 15.8 percent for fat, and 5.0 percent for ash were obtained when the results for nonfasted rats were calculated on a glycogen-free basis.[8] The percentage of protein and of ash in the livers of rats fed SP 8 HVO diet did not differ with age, but the percentage of fat showed a consistent tendency to increase. With SPE diet, as with stock diet, the composition of the liver appeared to be uninfluenced by the age of the animal. When the data for nonfasted rats fed this diet were calculated to a glycogen-free basis,[8] values for protein, fat, and ash were 55.8, 34.1, and 3.9 percent, respectively—values differing greatly from those on the stock diet.

Data comparing the *content* of the livers from fasted and nonfasted rats were limited but seemed to indicate that diet could influence the response of the liver to fasting. As the result of a 17-hour fasting period, the protein, fat, and ash content of the livers of rats fed SP 8 HVO diet were reduced. Both protein and ash content were smaller in the

[8] Assuming a liver glycogen of 8 percent on the dry-weight basis using a value obtained for comparable rats (unpublished data).

TABLE 56.—*Protein, fat, and ash in livers from rats maintaining weight at different ages on stock, SP 8 HVO, and SPE diets*

Diet and age of rats (days)	Rats	Average age	Average liver weight	Water	Based on dry weight						Total content		
					Protein		Fat		Ash				
					Average	Range	Average	Range	Average	Range	Protein	Fat	Ash
Stock:	*Number*	*Days*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*
Less than 300	9	224	10. 4	67. 1	76. 6	73. 3–80. 8	16. 9	13. 5–20. 7	4. 3	3. 5–4. 8	2. 61	0. 58	0. 14
300 to 499 [1]	7	413	16. 5	70. 1	70. 2	69. 0–72. 4	14. 9	13. 4–18. 0	4. 4	4. 0–4. 9	3. 70	. 73	. 22
500 and over [1]	6	580	20. 3	71. 8	70. 0	66. 7–72. 9	14. 3	14. 2–14. 6	4. 7	4. 5–4. 7	4. 04	. 84	. 27
SP 8 HVO:													
Less than 300	6	252	11. 0	68. 4	71. 9	67. 1–75. 0	17. 3	15. 5–21. 6	4. 6	4. 3–5. 1	2. 51	. 60	. 16
300 to 399	8	353	12. 0	67. 7	72. 6	67. 4–78. 2	18. 6	15. 3–21. 7	4. 6	4. 2–5. 1	2. 79	. 73	. 18
400 to 499	7	457	13. 4	68. 6	70. 7	67. 2–74. 9	19. 6	14. 8–23. 5	4. 5	4. 2–4. 7	2. 97	. 83	. 19
500 to 599	12	547	14. 1	67. 7	68. 9	62. 8–73. 8	21. 8	17. 4–28. 6	4. 6	4. 0–5. 1	3. 13	. 99	. 21
SPE:													
Less than 300	7	252	18. 2	59. 6	51. 3	41. 1–66. 3	43. 1	27. 0–52. 2	3. 1	2. 4–4. 0	3. 67	3. 33	. 22
Less than 300 [1]	4	276	29. 9	65. 1	51. 4	41. 2–61. 2	31. 4	17. 3–47. 4	3. 6	3. 0–4. 2	5. 26	3. 32	. 36
300 to 399	7	353	23. 0	59. 5	47. 5	40. 8–54. 4	45. 8	38. 1–50. 7	3. 4	2. 5–4. 1	4. 38	4. 30	. 31
400 to 499	10	453	21. 8	57. 0	45. 5	32. 6–64. 6	48. 8	29. 0–63. 3	3. 1	2. 4–3. 7	4. 11	4. 92	. 29
500 to 599	6	548	19. 9	60. 6	49. 3	39. 8–63. 3	43. 1	30. 8–55. 4	3. 4	2. 2–4. 0	3. 79	3. 49	. 26

[1] Nonfasted rats.

livers of fasted animals fed SPE diet than in those of nonfasted rats, but the fat content remained approximately the same whether or not the rats were fasted prior to sacrifice.

Although the percentage of liver fat among individual rats varied considerably, the livers from stock or SP 8 HVO rats were not considered fatty; those from SPE rats were consistently fatty. Only two of the animals fed SPE diet had livers with less than 30 percent fat. The highest percentage of fat observed was 63.3 percent in a liver from a rat fed SPE diet.

With the techniques used, fat was not detected microscopically unless present in excess of 30 percent. When the concentration of fat was between 30 and 40 percent, numerous small vacuoles were observed. When fat exceeded 40 percent, large as well as small vacuoles were apparent.

Although protein and ash in the livers from SPE rats were diluted by fat, the total content of each was equal to or greater than that found for these components in the livers of stock or SP 8 HVO rats.

In table 57 are summarized data on the composition of livers from rats fed stock, SP 8 HVO, and SPE diets as related to liver weight. There appeared to be little evidence that the composition of this organ was influenced by its size. Livers from stock rats were similar in composition whether their average weight was 14.9 or 23.1 grams. The percentage of fat in livers weighing less than 16 grams was low in comparison with the larger livers from rats fed SPE diet. However, small livers were rarely seen in rats fed this diet.

The increase in liver fat with age that was observed in rats fed SP 8 HVO diet seemed to be related, in part at least, to the body weight of these animals. In table 58 are summarized the results for liver fat as related to body weight. For animals weighing less than 600 grams, the increase in the average percentage of fat with age was small and, considering the range of values observed, was of questionable significance. The percentage of fat in the livers of rats weighing between 600 and 700 grams was significantly higher ($P < 0.01$) than in the livers of the lighter animals. Of the 11 rats weighing between 600 and 700 grams, 10 had livers containing more than 20 percent fat. In the larger rats weighing more than 700 grams, there was some indication that liver fat was lower when kidneys showed evidence of damage. The 4 rats in this group with liver fat less than 20 percent had kidneys showing degenerative changes; the 2 rats with normal kidneys

TABLE 57.—*Protein, fat, and ash in livers of different weights from fasted and nonfasted rats fed stock, SP 8 HVO, and SPE diets*

Condition, diet, and range of liver weights (grams)	Rats	Average liver weight	Water	Based on dry weight		
				Protein	Fat	Ash
Nonfasted rats:						
Stock:	*Number*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Less than 16.0	4	14. 9	71. 0	70. 4	15. 4	4. 3
16.0 to 19.9	5	16. 6	71. 7	68. 0	14. 3	4. 5
20.0 and over	5	23. 1	69. 9	69. 4	14. 8	4. 7
Fasted rats:						
SP 8 HVO:						
Less than 12.0	12	10. 7	67. 9	72. 0	19. 0	4. 6
12.0 to 13.9	10	13. 0	68. 4	71. 6	18. 7	4. 6
14.0 to 15.9	9	14. 6	67. 4	68. 2	22. 4	4. 6
16.0 and over	2	18. 0	70. 2	70. 2	18. 0	4. 7
SPE:						
Less than 16.0	3	14. 1	64. 6	64. 7	28. 9	3. 8
16.0 to 19.9	12	18. 2	60. 3	50. 9	42. 6	3. 3
20.0 and over	13	24. 1	59. 0	48. 4	45. 6	3. 3

TABLE 58.—*Liver fat and body weight of fasted rats maintaining weight on SP 8 HVO diet*

Body weight range (grams)	Rats	Average body weight	Average liver weight	Liver fat on dry-weight basis	
				Average	Range
	Number	*Grams*	*Grams*	*Percent*	*Percent*
Less than 500	6	462	10. 4	17. 1	14. 8–19. 1
500 to 599	10	550	12. 4	18. 0	15. 5–21. 7
600 to 699	11	653	13. 7	22. 1	19. 4–28. 6
700 and over	6	742	14. 6	21. 0	17. 8–28. 3

had livers containing 24.9 and 28.3 percent fat. The number of rats with damaged kidneys that were maintaining their weight on this diet was small, and more data are needed to establish the possible significance of this relationship.

On the stock diet, there was relatively small variation in body weight, and data for rats weighing more than 600 grams were too limited to determine whether or not there was any relationship between body weight and liver fat.

On SPE diet, with high concentrations of fat in the liver the usual finding, there was no evidence that the percentage of fat in the liver was related to body weight. A rat weighing 783 grams had a liver containing 38.6 percent fat; one weighing 530 grams had a liver containing 51.2 percent fat.

Rats losing weight on stock, SP 8 HVO, and SPE diets.—In table 59 are summarized data for rats that were losing weight on stock, SP 8 HVO, and SPE diets. The results are reported for nonfasted and fasted rats with further separation on the basis of the extent of weight loss even though the number of animals in some groups is small. Most of the results with the stock diet were for nonfasted rats and were similar to those observed with animals maintaining weight on this diet. When weight loss of nonfasted rats was less than 100 grams, the influence of glycogen on the percentage composition of the livers was apparent. Regardless of the extent of weight loss of fasted moribund rats fed SP 8 HVO diet, the composition of the liver was similar to that obtained for fasted rats that were maintaining weight. In nonfasted rats fed this diet, livers tended to contain a higher percentage of fat than in the fasted animals, but again there was little evidence that extent of weight loss influenced appreciably the composition of the livers. In contrast, the extent of weight loss before sacrifice for rats fed SPE diet seemed to influenced liver composition more than fasting. The fatty livers characteristic of rats that were maintaining weight on SPE diet were seen in many of the moribund rats fed this diet, although the percentage of fat was generally somewhat lower than in rats maintaining weight. When weight loss exceeded 100 grams, there was a marked decrease in the number of rats with liver fat exceeding 30 percent.

The wide variation in liver fat of nonfasted rats that were losing weight on SP 8 HVO diet did not appear to be merely a reflection of reduced food intake and extent of weight loss before sacrifice. Low liver fat in these rats seemed to be associated with excessively damaged kidneys, whether or not there had been appreciable weight loss. Data for liver fat and kidney damage are summarized in table 60. In the first group are included values for liver fat when kidney damage did not exceed a rating of 2 for hyalin casts, with no glomerular or cystic damage. In the second group are the results for rats with kidneys showing cystic and glomerular damage as well as hyalin. Of the 8 rats with kidneys showing little evidence of damage, 6 had livers containing more than 26 percent fat; only 1 of the 7 rats with extensive kidney damage had a liver containing more than 20 percent fat (21.5 percent). A similar trend has already been discussed for fasted SP 8 HVO rats that were maintaining weight. On SPE diet, high liver fats were found consistently in rats with kidneys showing little or no kidney damage; the highest fat was observed in a rat with small normal kidneys. However, high liver fats were also obtained frequently in rats with extensively damaged kidneys, and no consistent relation between fat in the liver and kidney damage was observed on this diet.

Rats maintaining weight on SPM, SPB, and SPPB diets.—In table 61 are summarized the more limited data for the composition of livers from rats fed SPM, SPB, and SPPB diets. Data are presented for two age groups: those 200 to 399 days, and those 400 to 599 days old. No marked differences were observed in the composition of the livers of the young rats fed these three diets, and the results are similar to those already reported for comparable animals fed SP 8 HVO diet. In the older rats, the percentage of fat in the livers was higher and the percentage of protein correspondingly lower than in 200- to 399-day-old animals. Liver fat for rats fed SPB diet was similar to that observed for comparable rats fed SP 8 HVO diet. Higher liver fats were obtained, however, for rats fed SPM or SPPB diets. The percentage of ash in these livers was similar to that found in the livers of SP 8 HVO rats, and no differences with age were observed.

The high liver fats for rats fed SPM and SPPB diets appear to be related to their body weight. Differences in body weight, however, do not explain the results for rats fed SPB diet, as seen in table 62. On the diet containing milk (SPM), liver fat increased on the average from 16.9 for rats weighing less than 500 grams to 29.7 for those weighing 700 grams and more. A similar trend was seen with rats fed the diet containing peanut butter (SPPB). On beef (SPB), however, only 3 of the 13 rats had livers containing more than 20 percent fat, with one of the highest values observed in a rat weighing 486 grams.

Rats losing weight on SPM, SPB, and SPPB diets.—No consistent trend with age was apparent from the data on the composition of the livers from rats that were losing weight on SPM, SPB, or SPPB diets. Therefore, the data in table 63 are the results for all age groups combined. The livers from these moribund rats showed differences in composition that were similar to those from rats that were maintaining weight. Liver fat was consistently low for SPB rats, both fasted and nonfasted. Liver fat tended to be high in SPM rats, and again the high fat values seemed to be related to the large number of heavy animals on this diet. The highest liver fat with SPM diet was 47 percent in a rat that lived to be 799 days old and reached a maximum weight of 1,020 grams.

TABLE 59.—*Protein, fat, and ash in livers from fasted and nonfasted rats losing weight on stock, SP 8 HVO, and SPE diets*

Condition, weight loss, and diet	Rats	Average age	Average liver weight	Water	Based on dry weight: Protein, Average	Protein, Range	Fat, Average	Fat, Range	Ash, Average	Ash, Range	Total content: Protein	Fat	Ash
Fasted rats													
Losing less than 100 grams:	*Number*	*Days*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*
Stock	1	757	11.8	70.6	77.5	77.5	17.8	17.8	5.1	5.1	2.68	0.62	0.18
SP 8 HVO	6	564	11.7	69.6	71.5	66.9–77.8	17.9	14.1–20.5	4.7	4.6–5.2	2.52	.65	.17
SPE	18	391	17.5	63.4	57.9	35.5–74.2	34.8	15.3–60.3	3.8	1.7–5.1	3.56	2.47	.23
Losing more than 100 grams:													
SP 8 HVO	3	550	15.0	69.2	71.5	69.2–73.1	16.2	15.7–16.6	4.8	4.7–4.8	3.32	.75	.22
SPE	8	422	19.5	67.7	67.2	52.2–81.2	26.3	14.5–20.9	4.6	4.0–5.2	4.12	1.78	.29
Nonfasted rats													
Losing less than 100 grams:													
Stock	2	854	22.6	70.2	69.0	68.7, 69.2	13.6	12.7, 14.1	4.4	4.2, 4.5	4.65	.91	.30
SP 8 HVO	6	384	17.7	68.8	64.7	57.8–75.8	21.8	17.5–26.6	4.1	3.4–4.7	3.53	1.17	.22
SPE	3	226	20.4	57.9	47.6	33.1–54.8	45.4	38.1–60.0	3.3	2.3–3.8	3.76	4.49	.26
Losing more than 100 grams:													
Stock	4	626	17.7	71.4	78.8	75.6–80.2	14.5	13.6–15.6	5.1	4.7–5.4	3.99	.74	.26
SP 8 HVO	9	765	16.0	69.1	68.8	57.1–79.6	21.6	14.9–37.6	4.3	3.4–4.8	3.34	1.03	.21
SPE	8	385	21.8	66.2	61.1	42.4–75.4	29.9	15.3–53.7	4.0	3.0–4.9	4.35	2.49	.29

TABLE 60.—*Kidney damage and liver fat in nonfasted rats losing weight on SP 8 HVO diet*

Kidney damage	Rats	Rating for kidney damage [1]			Liver fat on dry-weight basis
		Hyalin	Cystic	Glomerular	
	Number	*Score*	*Score*	*Score*	*Percent*
0 to 2.0 hyalin, no cystic or glomerular damage	8	0.4	0	0	25.6
2.0 hyalin or more, with cystic and glomerular damage	7	3.0	2.4	2.3	18.5

[1] Based on maximum rating of 4.

Liver fats tended to be high in nonfasted SPPB rats.

RATS FED OTHER EXPERIMENTAL DIETS.—In table 64 are summarized the data available on the composition of livers from rats fed some of the other experimental diets. The results are chiefly for moribund rats, and interpretation is complicated by the variable weight loss of these animals before death. In spite of the wide range of values obtained, some differences were observed that seemed to be related to diet. On the various diets that consisted of the SPE diet with purified supplements, liver fats were generally high and similar to the results with the unsupplemented diet. When the diet contained cholesterol as the supplement, however, liver fat tended to be somewhat higher than for the other modifications of SPE diet, in agreement with the results already discussed for microscopic examinations of this organ for fat.

In the series to determine the influence of kind and/or level of fat, the livers from rats fed SP 16 HVO tended to contain a higher percentage of fat than those from rats on SP 8 HVO. Livers were not excessively fatty with any of these diets, and no consistent differences were observed that related to the level or kind of dietary fat. When diets of 100 percent whole egg or egg yolk were fed, liver fats tended to be low and protein correspondingly high in comparison with the liver fat of rats fed a diet containing 25 percent whole egg. The results for rats fed 100 percent whole egg and 100 percent egg yolk were similar to each other. The results for a small group of littermates showing little or no weight loss on SPE, Y100, and Y97+ salt diets confirmed the finding that liver fat was lower with 100 percent egg yolk than with SPE diet. Supplementation of the 100 percent egg yolk with salt mixture was without influence on liver fat. The livers of Wistar rats fed SP 8 HVO diet were generally low in fat; 18.4 percent was the highest value obtained. The only high liver fat (42 percent) observed in Wistar rats was for a rat 874 days old that reached a maximum weight of 970 grams on SPE diet.

DISCUSSION.—Liver fats have been reported to be susceptible to many factors, including age, heredity, and diet. Much of the research dealing with liver lipids has been done on relatively young animals, and little information is available on the liver lipids of the rat throughout life. Andrew, Shock, Barrows, and Yiengst (*11*) reported the results of histological examination of the livers from stock animals 1 and 2 years of age. The two groups of rats were very much alike. Vacuolation indicating fat storage was observed occasionally, but no consistent change with age was apparent. Periportal infiltration of lymphocytes was seen in the connective tissue around the bile ducts, portal vein, and hepatic artery in some of the older rats. From chemical analysis, Yiengst, Barrows, and Shock (*191*) reported no differences in fat content of the livers from these two groups of rats. Grunt, Berry, and Knisely (*80*), and Grunt and Knisely (*81*) reported that old animals have more hepatic fat than have the young ones, and that genetic factors appear to play a significant role in the development of fatty livers in the rat.

Literature dealing with the many dietary factors that may produce fatty livers has been extensively reviewed by Deuel (*47*). Publications on this subject are numerous, and only a few that seem closely related to the results under consideration in this bulletin will be discussed.

Blatherwick, Medlar, Bradshaw, and others (*31*) fed rats diets containing 75 percent dried liver for periods of 30 and 60 days, and obtained livers of high fat and cholesterol content. Fat accumulation was well marked by 28 days. Fractions from liver were fed alone or with various supplements, including lecithin and cholesterol. Marked differences in the fat and cholesterol content of the livers were noted, but changes in liver fat were not necessarily paralleled by changes in liver cholesterol. Cholesterol, when added to a diet containing a 70 percent alcoholic precipitate of an aqueous extract of liver, resulted in livers of extremely high fat and cholesterol content. Feeding certain fractions containing cholesterol, however, had relatively little effect. The feeding of cooked egg yolks produced livers with increased cholesterol content and, to a less degree, increased fat. More marked increases were observed with cooked whole eggs.

McCay, Maynard, Sperling, and Osgood (*121*) reported higher total lipids, cholesterol, and phospholipids in the liver of rats fed dried liver than in those fed milk proteins. The lipid composition of the livers of these animals was about

TABLE 61.—*Protein, fat, and ash in livers from fasted rats maintaining weight on SPM, SPB, and SPPB diets*

Diet and age of rats (days)	Rats	Average age	Average liver weight	Water	Based on dry weight						Total content		
					Protein		Fat		Ash				
					Average	Range	Average	Range	Average	Range	Protein	Fat	Ash
SPM:	*Number*	*Days*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*
200 to 399	6	304	12. 3	67. 8	72. 8	67. 2—76. 3	19. 2	16. 7—26. 1	4. 3	4. 1—4. 6	2. 86	0. 78	0. 17
400 to 599	8	504	13. 9	66. 7	67. 2	55. 2—72. 8	24. 6	18. 8—33. 2	4. 2	3. 6—4. 5	3. 08	1. 19	. 19
SPB:													
200 to 399	6	305	11. 8	68. 9	74. 4	71. 6—75. 2	18. 0	15. 9—20. 7	4. 2	3. 3—4. 5	2. 74	. 67	. 15
400 to 599	7	503	13. 9	68. 9	70. 7	66. 4—74. 5	20. 5	16. 8—26. 8	4. 5	4. 0—5. 5	3. 06	. 83	. 20
SPPB:													
200 to 399	5	295	12. 2	67. 8	73. 6	67. 8—78. 0	18. 8	15. 0—22. 4	4. 0	3. 7—4. 5	2. 88	. 75	. 15
400 to 599	11	522	17. 2	67. 4	66. 3	62. 2—71. 3	24. 9	20. 5—29. 6	4. 1	3. 5—4. 7	3. 68	1. 40	. 23

TABLE 62.—*Liver fat in rats of different body weight maintaining weight on SPM, SPB, and SPPB diets*

Diet and body weight (grams)	Rats	Average body weight	Liver fat on dry-weight basis
	Number	*Grams*	*Percent*
SPM:			
Less than 500	2	434	16. 9
500 to 599	3	575	20. 8
600 to 699	4	659	23. 0
700 and over	2	774	29. 7
SPB:			
Less than 500	3	441	19. 6
500 to 599	2	549	20. 1
600 to 699	7	654	19. 0
700 and over	1	740	19. 0
SPPB:			
Less than 500	3	444	17. 3
500 to 599	1	550	19. 7
600 to 699	5	667	23. 6
700 and over	7	777	25. 5

the same whether the diets contained 10 or 41 percent liver.

Reussner and Thiessen (*156*) reported 29.3 percent fat in the large livers from rats fed an egg and bacon diet, in contrast to 12.5 percent for a cereal and milk diet and 15.1 percent for a milk diet. These differences in liver fat were not related to the level of fat in the diet. In spite of the high liver fat, the bacon and egg diet resulted in good survival of rats on long-term feeding.

Okey (*140*) fed diets containing 1 percent cholesterol to rats from weaning throughout middle and old age, and observed no significant differences in growth, health, and survival between control and cholesterol-fed rats in spite of the high fat and cholesterol content of the livers of the latter. Histological examination of the livers showed fatty infiltration rather than degeneration of functioning tissues.

Ridout, Lucas, Patterson, and Best (*157*) reported a progressive increase with increasing dietary cholesterol in the accumulation of both glycerides and cholesterol esters in the livers of rats.

Okey and Lyman (*143*) reported the results of feeding 5, 10, and 15 percent coconut oil or cottonseed oil with or without added cholesterol. The response of female rats was occasionally quite different from males and, for comparison with the results reported in this publication, only the results with males will be discussed. In the absence of dietary cholesterol, elevated liver fats were observed only when coconut or cottonseed oil was fed at the 15-percent level. In the presence of cholesterol, liver lipids and total cholesterol increased with increasing concentration of dietary fat and were consistently higher with cottonseed oil than with coconut oil.

Okey, Lyman, Harris, and others (*145*) also compared the response of rats to 13 different fats with iodine numbers varying from 8 to 143, each fed at the 10-percent level. Liver fat and cholesterol values in the absence of dietary cholesterol were not exceedingly high with any of the 13 edible fats investigated. There was a tendency to relatively low values with the more highly saturated fats. When cholesterol was included in the diet, liver fat and cholesterol increased; the increase in cholesterol was generally smallest in rats fed fats with low iodine numbers. According to the author, not one but a number of factors appear to influence the effect of composition of dietary fat on liver lipids.

Choline has been found effective in preventing various types of fatty livers in rats (*24*). In a diet free from cholesterol, Best, Lucas, Patterson, and Ridout (*23*) reported that regardless of the kind of fat used, total liver lipids were essentially normal when the amount of choline chloride present was between 0.12 and 0.16 percent. According to Benton, Harper, and Elvehjem (*19*), the type of dietary fat had little effect on the deposition of liver fat when the diet of the rat contained adequate amounts of choline and protein. Ridout, Lucas, Patterson, and Best (*157*) found that the amount of choline needed to maintain liver glycerides within or slightly above normal when the diet contains cholesterol does not necessarily prevent the accumulation of cholesterol esters in the liver.

Jackson (*100*) observed a tendency to fatty livers with degenerative abnormalities when rats were fed a diet containing 80 percent sucrose. This diet, however, did not appear detrimental to growth and general health. On diets containing 45 percent sucrose or starch, no marked differences were observed in size or histological structure of livers or kidneys.

SUMMARY.—The results with BHE rats reported in this publication indicate that age may or may not be a factor in the percentage of fat in the liver, depending on the diet under investigation. The increase that occurred with age on some of the diets appeared to be related to an increase in the number of extremely heavy animals on these diets. Differences in liver fat were not necessarily related to level of dietary fat. The percentage of fat in the diet containing beef was somewhat higher than that in the diets containing egg, milk, or peanut butter, but liver fat for rats fed this diet was generally low when compared with the results for the other diets. Liver fats were high in rats fed the diet containing 25 percent egg and much higher than were obtained with the extremely high fat diet consisting of 100 percent egg yolk. The results with rats fed 8 or 16 percent HVO, lard, or butter also showed no consistent relation between level of dietary fat and liver fat, although these results were complicated by the weight loss occurring in moribund rats.

Damaged kidneys were a frequent finding in BHE rats regardless of diet, and they were not

TABLE 63.—*Protein, fat, and ash in livers from fasted and nonfasted rats losing weight on SPM, SPB, and SPPB diets*

Condition and diet of rats	Rats	Average age	Average liver weight	Water	Based on dry weight						Total content		
					Protein		Fat		Ash				
					Average	Range	Average	Range	Average	Range	Protein	Fat	Ash
	Number	*Days*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*
Fasted rats:													
SPM	4	398	14. 5	65. 5	61. 4	58. 6–67. 8	29. 6	27. 0–31. 3	3. 6	2. 3–4. 3	3. 05	1. 51	0. 19
SPB	4	427	12. 8	68. 1	71. 1	70. 2–73. 8	18. 0	15. 2–18. 9	4. 5	4. 3–4. 5	2. 90	. 72	. 18
SPPB	9	425	18. 9	68. 4	66. 9	58. 3–74. 4	19. 5	15. 0–23. 8	4. 6	3. 8–5. 4	3. 95	1. 17	. 28
Nonfasted rats:													
SPM	10	580	17. 1	69. 2	59. 5	34. 8–73. 1	25. 3	15. 9–47. 7	3. 9	2. 3–5. 0	3. 06	1. 43	. 20
SPB	4	358	17. 3	69. 4	67. 0	60. 1–75. 2	18. 8	15. 0–23. 2	4. 2	3. 8–4. 6	3. 51	1. 07	. 22
SPPB	5	511	22. 2	73. 3	60. 8	56. 5–64. 4	23. 8	15. 9–29. 4	4. 5	4. 0–5. 2	3. 71	1. 36	. 27

TABLE 64.—*Protein, fat, and ash in livers from rats fed other experimental diets*

Strain and diet	Rats	Average age	Average weight loss	Average liver weight	Water	Based on dry weight: Protein	Based on dry weight: Fat	Based on dry weight: Ash	Total content: Protein	Total content: Fat	Total content: Ash
BHE rats											
SPE supplemented with—	*Number*	*Days*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*
Choline, 0.5%	19	492	139	21.2	66.2	62.2	26.6	4.2	4.24	2.25	0.28
B_{12}, 0.01 mg./100 gm	8	463	131	21.0	67.2	65.2	24.8	4.2	4.35	1.96	.28
Choline, 0.5%+B_{12}, 0.01 mg./100 gm	10	434	114	24.9	68.4	65.4	23.9	4.4	4.98	2.01	.33
B_6, 0.5 mg./100 gm	8	472	128	20.3	64.0	58.8	29.8	4.1	4.14	2.41	.30
Choline, 0.5%+B_6, 0.5 mg./100 gm	9	412	127	19.6	64.8	58.2	31.2	3.9	3.92	2.29	.27
Choline, 0.5%+B_{12}, 0.01 mg./100 gm.+B_6, 0.5 mg./100 gm	10	431	129	19.9	64.0	59.4	29.7	4.7	4.09	2.31	.33
Cholesterol, 0.46%	8	451	150	23.4	64.0	56.2	36.0	4.2	4.58	3.21	.34
Cholesterol, 1.38%	10	409	142	22.2	61.6	53.3	39.5	3.8	4.20	3.84	.32
Ascorbic acid, 0.2%	8	436	130	19.2	67.1	65.6	25.0	3.8	3.92	1.95	.23
Ascorbic acid, 0.2%+cholesterol, 0.46%	9	434	183	20.8	66.1	64.4	27.8	4.3	4.25	2.60	.29
SP 16 HVO	8	629	84	16.3	68.6	65.0	24.9	4.4	3.26	1.27	.22
SP 8 lard	10	548	84	15.5	68.1	67.2	22.8	4.7	3.31	1.14	.23
SP 16 lard	9	572	117	15.6	68.9	69.4	19.3	4.6	3.37	.94	.22
SP 8 butter	9	563	87	14.1	67.6	66.2	21.9	4.6	3.02	1.00	.21
SP 16 butter	9	486	87	14.8	67.3	65.2	23.9	4.5	3.14	1.16	.22
E100	8	568	145	17.2	70.7	64.0	21.4	4.3	3.19	1.10	.22
Y100	14	375	80	14.6	68.1	63.1	24.4	4.5	2.93	1.14	.21
Littermates fed—											
SPE	5	428	66	21.5	63.4	59.5	38.8	3.6	4.60	3.20	.28
Y100	5	425	35	17.3	69.3	74.0	22.6	3.6	3.81	1.18	.18
Y97+salt mixture, 3.0%	4	424	6	14.1	67.0	70.3	25.3	2.8	3.25	1.19	.13
Diet reversal:											
Sacrificed at approximately 250 days:											
Stock	3	250	0	17.0	-----	-----	-----	-----	-----	-----	-----
SPE	3	249	0	26.4	76.0	54.3	27.8	3.3	3.40	1.72	.21
Continued on—											
Stock	3	590	195	18.5	71.1	71.5	16.0	-----	3.82	.85	-----
SPE	2	396	133	16.7	71.5	77.0	18.1	4.3	3.62	.85	.20
Reversed at 250 days:											
Stock changed to SPE	4	686	109	22.3	67.7	59.5	24.7	3.9	4.17	1.81	.28
SPE changed to stock	3	577	166	21.7	70.9	68.9	15.7	4.4	4.35	.98	.28
Wistar rats											
SP 8 HVO	8	762	119	13.7	72.2	70.5	17.1	4.3	2.67	.64	.16
SPE	5	772	103	17.8	70.2	69.9	22.1	4.4	3.64	1.19	.23

necessarily accompanied by fatty livers. There was some indication with other than SPE diets that large livers tended to be associated with kidney damage and that changes in the liver with age might be important in the production of kidney damage even though the liver itself showed no evidence microscopically of damage. On SPE diet alone or with added supplements, liver fat was high at a relatively early age, often in rats with apparently normal kidneys. The infiltration of the liver with fat that was observed with SPE rats may, however, accelerate kidney changes. The tendency to enlarged, damaged kidneys on all diets suggested an inherent kidney weakness in the BHE strain of rats. It is conceivable that an excessive load may be placed on the kidneys because of improper functioning of the liver resulting from some defect in certain enzyme systems responsible for the normal activity of this organ.

Differences in the accumulation of fat in the livers of BHE and Wistar rats when fed SPE diet under identical conditions provide evidence suggesting inherent differences in the metabolic processes involved in the utilization of this diet by these two strains of rats.

Serum cholesterol

Serum cholesterol measurements were usually made on the blood from fasted rats, and only the results with fasted animals are included in this section.

Rats maintaining weight on stock, SP 8 HVO, and SPE diets.—The influence of age and diet on cholesterol levels in the sera from rats maintaining weight on stock, SP 8 HVO, and SPE diets is summarized in table 65. Age appeared to exert no influence on the serum cholesterol levels or rats fed *stock diet*. A cholesterol level of 114 mg./100 ml. was obtained for a 976-day-old rat fed this diet. Cholesterol values were below 150 mg./100 ml. in 80 percent of the sera analyzed. On *SP 8 HVO diet*, serum cholesterol levels were similar to those on stock diet when the age of the rats was less than 500 days. In rats more than 500 days old, however, only 43 percent of the cholesterol values were below 150 mg./100 ml. and 3 of the animals in this group had sera containing more than 200 mg./100 ml. On *SPE diet*, serum cholesterol levels were high even in the youngest group of rats, and were consistently higher than 150 mg./100 ml. for all age groups. Values exceeding 200 mg./100 ml. were a frequent finding, and 9 of these SPE rats had serum levels exceeding 300 mg./100 ml. The high serum cholesterol levels in rats 300 to 399 days of age seem to be another indication that this period is a critical one in the response of rats to SPE diet.

Table 65.—*Influence of diet and age on serum cholesterol levels in rats maintaining weight on stock, SP 8 HVO, SPE, SPM, SPB, and SPPB diets*

Diet and age of rats (days)	Rats	Average age	Serum cholesterol: Average	Range	Rats with cholesterol levels of—: Less than 150 mg./100 ml.	150 to 199 mg./100 ml.	200 to 299 mg./100 ml.	300 mg./100 ml. and over
	Number	*Days*	*Mg./100 ml.*	*Mg./100 ml.*				
Stock:								
Less than 300	19	229	120	79–217	79	16	5	0
300 to 499	5	410	117	97–151	80	20	0	0
500 and over	6	691	127	107–179	83	17	0	0
SP 8 HVO:								
Less than 300	6	252	103	90–117	100	0	0	0
300 to 399	8	353	122	81–185	75	25	0	0
400 to 499	6	457	131	112–154	83	17	0	0
500 to 599	14	546	166	99–270	43	36	21	0
SPE:								
Less than 300	7	252	199	157–240	0	57	43	0
300 to 399	7	353	351	178–670	0	14	43	43
400 to 499	14	455	232	152–402	0	43	43	14
500 to 599	11	530	287	197–432	0	9	55	36
SPM:								
Less than 400	6	304	118	102–128	100	0	0	0
400 to 599	6	512	164	87–265	50	17	33	0
SPB:								
Less than 400	6	304	103	80–152	83	17	0	0
400 to 599	6	513	163	79–243	50	17	33	0
SPPB:								
Less than 400	6	289	135	95–236	83	0	17	0
400 to 599	11	530	221	129–343	9	36	36	18

RATS MAINTAINING WEIGHT ON SPM, SPB, AND SPPB DIETS.—The limited data for rats fed diets containing high levels of milk, beef, or peanut butter are reported in table 65. The results for rats fed *SPM* and *SPB* diets were similar to those for SP 8 HVO rats. In general, serum cholesterol levels were low in rats under 400 days of age, and tended to be higher in rats 400 to 599 days of age. The slightly higher value for rats under 400 days of age on *SPPB* diet was due to inclusion of one rat with a value of 236 mg./100 ml., and is of questionable significance considering the limited data available. In the older rats fed this diet, however, cholesterol values were significantly higher ($P<0.01$) than those for rats of comparable age fed stock, SP 8 HVO, SPM, or SPB diets, with 54 percent exceeding 200 mg./100 ml.

RATS LOSING WEIGHT ON STOCK, SP 8 HVO, SPE, SPM, SPB, AND SPPB DIETS.—In table 66 are summarized similar data for rats that were losing weight on these same diets. The results are reported without regard to weight loss before sacrifice. Although there seemed to be a trend toward somewhat higher cholesterol values when weight loss exceeded 100 grams, the data available were too few to warrant separation on this basis. Differences in serum cholesterol with age and with diet were still apparent in the moribund rats. Cholesterol levels exceeding 150 mg./100 ml. were rarely seen in rats less than 400 days old on SP 8 HVO, SPM, and SPB diets. In the intermediate age group, elevated cholesterol values were found with all diets, although the proportion of rats with high serum levels and the extent of the elevation depended upon the dietary regimen of the rats. Except with SPPB diet, rats with a relatively long lifespan tended to have lower cholesterol levels than did those in the intermediate age group. Rats fed stock or SP 8 HVO diets generally had lower levels than did those fed the other diets. One exceedingly high value of 1,813 mg./100 ml. has been omitted from the average value recorded for stock rats. In this animal the thyroid gland was almost entirely replaced by tumor. On SPE and SPPB diets, the proportion of elevated cholesterol values was high in comparison with those obtained in rats fed the other diets investigated.

CHOLESTEROL AND KIDNEY DAMAGE.—The increase in serum cholesterol with age appeared to be associated, in part at least, with an increase in the number of rats with enlarged and damaged kidneys. In table 67 are summarized data relating kidney size to serum cholesterol level. On stock, SP 8 HVO, SPM, or SPB diets, levels exceeding 150 mg./100 ml. were rarely found in healthy or moribund rats with kidneys weighing less than 1.8 grams. Kidneys in this weight range showed little evidence of degenerative change. Somewhat higher serum cholesterol levels tended

TABLE 66.—*Influence of diet and age on serum cholesterol levels in rats losing weight on stock, SP 8 HVO, SPE, SPM, SPB, and SPPB diets*

Diet and age of rats (days)	Rats	Average age	Serum cholesterol					
			Average	Range	Rats with cholesterol levels of—			
					Less than 150 mg./100 ml.	150 to 199 mg./100 ml.	200 to 299 mg./100 ml.	300 mg./100 ml. and over
	Number	*Days*	*Mg./100 ml.*	*Mg./100 ml.*				
Stock:								
500 and over	9	721	166	101–235	56	22	22	0
SP 8 HVO:								
Less than 400	5	264	91	80–103	100	0	0	0
400 to 699	12	570	182	84–385	42	33	8	17
700 and over	3	743	113	96–129	100	0	0	0
SPE:								
Less than 300	7	189	153	90–248	57	0	43	0
300 to 399	16	367	386	121–904	6	6	19	69
400 to 599	40	497	346	132–932	5	2	35	58
600 to 699	12	640	218	115–290	17	0	83	0
SPM:								
Less than 400	4	340	184	103–419	75	0	0	25
400 to 599	5	501	258	122–573	20	20	40	20
600 and over	1	600	175	175	0	100	0	0
SPB:								
Less than 400	3	268	118	98–134	100	0	0	0
400 to 599	3	535	348	155–620	0	33	33	33
600 and over	5	704	162	105–220	40	20	40	0
SPPB:								
Less than 400	12	306	163	51–298	67	8	25	0
400 to 599	10	520	276	138–654	30	10	30	30
600 and over	2	663	315	289, 341	0	0	0	100

Table 67.—*Serum cholesterol levels of rats with kidneys of different weights on stock, SP 8 HVO, SPE, SPM, SPB, and SPPB diets*

Diet	Rats	Kidneys less than 1.80 grams		Rats	Kidneys 1.80 to 2.19 grams		Rats	Kidneys 2.20 grams and over	
		Average weight	Average cholesterol		Average weight	Average cholesterol		Average weight	Average cholesterol
	Number	*Grams*	*Mg./100 ml.*	*Number*	*Grams*	*Mg./100 ml.*	*Number*	*Grams*	*Mg./100 ml.*
Stock	31	1. 49	108	6	1. 94	142	4	2. 78	225
SP 8 HVO	34	1. 47	114	10	1. 95	157	10	3. 88	228
SPE	27	1. 52	192	10	1. 91	280	76	5. 51	343
SPM	13	1. 33	115	7	1. 95	187	3	3. 06	397
SPB	15	1. 44	113	1	1. 86	241	8	3. 32	302
SPPB	18	1. 46	147	13	1. 99	222	10	4. 45	304

to accompany the small normal kidneys from rats fed SPPB diet. The highest levels to accompany kidneys in this weight range were those from rats fed SPE diet. Kidneys weighing between 1.8 to 2.2 grams frequently showed some signs of damage, chiefly evidenced by the presence of hyalin casts. Cholesterol levels accompanying kidneys in this weight range were consistently higher than those found in rats with small kidneys. Cholesterol values in rats with badly damaged kidneys exceeding 3.0 grams in weight were generally high, but neither kidney size nor the types of kidney damage observed showed any quantitative relationship to the extent that the serum cholesterol was elevated.

A further consideration of the cholesterol data suggests that the influence of age on serum levels may be related chiefly to the increase with age in the number of rats with enlarged damaged kidneys. Evidence for this is seen in the data summarized in table 68 for rats fed the semipurified diet, where the results for rats with damaged kidneys have been excluded. The main difference between the results in this table and in table 65 lies in the results for rats 500 days and older. The 5 animals in this age group with small normal kidneys had an average cholesterol value of 120 mg./100 ml. similar to that found for younger rats, in contrast to 166 mg./100 ml. (table 66) for all 14 animals in this age group.

Table 68.—*Serum cholesterol levels of rats with normal kidneys at different ages on SP 8 HVO diet*

Average age (days)	Rats	Cholesterol	
		Average	Range
	Number	*Mg./100 ml.*	*Mg./100 ml.*
252	6	103	90–117
354	6	119	84–164
460	5	133	112–154
549	5	120	99–150

Serum cholesterol in littermates.—No consistent relation of serum cholesterol to liver size, liver fat, and adrenal or thyroid size was observed. There was some indirect evidence that the wide range of serum cholesterol levels was due in part to differences in inherited characteristics in the mixed strain of rats under investigation. Littermates were used in each of the experimental series for comparison of the various dietary regimens, but relatively few data were available to compare the response of littermates fed the same experimental diet. One series with SPE diet provided some data that permitted a direct comparison of the response of littermates to this diet under strictly comparable conditions. In table 69 are summarized the findings for two littermates from each of four litters. All were sacrificed at about 550 days of age. Data are included not only for serum cholesterol levels but also for the organ weights of these rats. Only one of the animals had lost more than 100 grams when sacrificed. Although the range of serum cholesterol values for the individual rats in the four litters was wide, varying from 152 to 432 mg./100 ml., the serum levels for littermates were relatively close. In contrast, organ weights of individual rats varied considerably even for littermates.

Rats fed other experimental diets.—In table 70 are summarized limited data on serum cholesterol levels in rats fed the other experimental diets. Most of the data were for sick or moribund rats that were losing weight at the time of sacrifice.

The wide range of values and the tendency to elevated serum cholesterol values observed in SPE rats were also apparent in rats fed the various supplemented SPE diets. No significant differences were observed in the serum cholesterol levels on any of these diets. Most of these rats had lost more than 100 grams before sacrifice and had large damaged kidneys. A comparison of the serum levels in rats that were maintaining weight might show differences not apparent in these moribund animals.

When the level of HVO was increased from 8 to 16 percent, the average serum cholesterol level

Table 69.—*Serum cholesterol levels and organ weights for rat littermates fed SPE diet*

Litter No.	Identification No.	Age	Maximum weight	Weight at death	Serum cholesterol	Organ weights			
						Liver	Kidney	Adrenal	Thyroid
		Days	*Grams*	*Grams*	*Mg./100 ml.*	*Grams*	*Grams*	*Mg.*	*Mg.*
7	1	513	714	611	267	19.1	4.73	46	26
	2	513	675	657	275	26.6	2.58	27	21
8	1	510	530	516	285	16.9	1.76	18	15
	2	511	590	585	220	13.5	2.32	24	20
10	1	504	580	560	432	25.0	2.32	18	18
	2	504	535	500	362	18.6	1.88	17	15
13	1	494	563	542	154	16.4	4.50	27	19
	2	494	566	511	152	16.8	6.37	29	27

Table 70.—*Serum cholesterol levels in rats fed other experimental diets*

Weight status and diet of rat	Rats	Average age	Serum cholesterol	
			Average	Range
Losing weight on—				
SPE supplemented with—	*Number*	*Days*	*Mg./100 ml.*	*Mg./100 ml.*
Choline, 0.5%	11	483	365	264–700
B_{12}, 0.01 mg./100 gm	4	549	296	221–440
Choline, 0.5%+B_{12}, 0.01 mg./100 gm	3	562	291	204–382
B_6, 0.5 mg./100 gm	5	512	346	252–510
Choline, 0.5%+B_6, 0.5 mg./100 gm	6	431	334	257–457
Choline, 0.5%+B_{12}, 0.01 mg./100 gm.+B_6, 0.5 mg./100 gm	7	447	344	196–549
Cholesterol, 0.46%	8	451	351	261–452
Cholesterol, 1.38%	7	422	370	210–470
Ascorbic acid, 0.2%	5	460	425	224–930
Ascorbic acid, 0.2%+cholesterol, 0.46%	7	431	421	164–840
SP 16 HVO	6	679	138	96–209
SP 8 lard	10	548	282	137–674
SP 16 lard	6	591	285	198–455
SP 8 butter	6	626	204	156–294
SP 16 butter	6	529	168	118–209
Y100	10	420	186	115–311
Maintaining weight on—				
Y100	4	461	213	176–252
Y97+salt mixture, 3.0%	5	430	190	169–218
SPW 8 HVO	6	550	110	80–158

(138 mg./100 ml.) was similar to that observed on the lower level of this fat. With butter or lard there was no evidence that increasing the level of the fats caused any appreciable change in serum cholesterol. Serum cholesterol values, however, tended to be much higher when the dietary fat was lard than when it was HVO.

Serum cholesterol values were obtained for rats on only two of the diets investigated to determine the influence of egg or egg fractions—SPW 8 HVO and E100. On SPW 8 HVO diet, cholesterol levels were similar to those for comparable SP 8 HVO rats. On the diet consisting solely of egg yolk, serum values were significantly lower than when the diet contained 25 percent whole egg. Supplementation of egg yolk with salt mixture had little influence on serum cholesterol.

Discussion.—The results reported in this publication as well as those reported by other investigators have shown that many factors influence cholesterol levels in the blood, and that the interactions between dietary factors and other factors such as heredity, age, and sex make the problem of interpreting serum values a difficult one. Many review articles have appeared dealing with various aspects of cholesterol metabolism. Kritchevsky's (*109*) book provides information on the biological significance and function of cholesterol. Portman and Stare (*153*) reviewed the many factors important in the dietary regulation of serum cholesterol levels. Deuel (*46*) covered many phases of lipid metabolism in relation to blood cholesterol levels.

Discussion of the literature dealing with serum cholesterol will be limited to those investigations

that seem most closely related to the results reported in this bulletin. Blood cholesterol levels vary considerably among species and only reports dealing with rat as the experimental animal will be considered, along with a few of the reports dealing with humans because of interest in the possible application to humans of the results obtained with rats. The results for rats will be confined to those reported for male rats.

The response of serum cholesterol levels to diet often differs, depending upon the absence or presence of cholesterol in the diet. Comparison of serum cholesterol values, therefore, must take into account whether we are dealing with endogenous cholesterol or with serum cholesterol levels that may be reflecting both endogenous and exogenous cholesterol.

The liver is the chief source of *endogenous cholesterol* and not only is able to synthesize this sterol but also is active in its breakdown and excretion. Serum cholesterol values in the absence of dietary cholesterol generally reflect the balance of activity in the liver with regard to these two processes.

With humans, serum cholesterol levels reflect lifetime dietary habits which generally include the consumption of cholesterol-containing foods. With the rat, however, many investigations deal with diets that contain little or no cholesterol so that blood cholesterol is strictly endogenous in origin. Even in the absence of dietary cholesterol, the results relating the kind and/or level of fat to serum cholesterol levels of rats are not entirely consistent.

In the *absence of dietary cholesterol*, several investigators report a tendency for blood cholesterol levels in rats to increase with increasing unsaturation of dietary fat. Klein (*105*), feeding diets containing 5- and 30-percent levels of Crisco or corn oil found plasma cholesterol levels to increase as the intake of linoleic acid increased. Swell, Flick, Field, and Treadwell (*179*) reported increased levels with increasing unsaturation of fat when rats were fed diets containing soybean fat hydrogenated to different iodine values. Nath, Wiener, Harper, and Elvehjem (*136*) reported little effect on serum cholesterol levels in rats as the result of feeding increasing quantities of hydrogenated coconut oil but a slight increase when 1 percent corn oil was added to the diet. Sunflower seed oil (*79*) has also been reported to elevate blood cholesterol.

With diets in which sucrose was the carbohydrate, Marshall, Hildebrand, Dupont, and Womack (*126*) obtained significantly higher cholesterol levels with 15 percent corn oil than with 3 percent corn oil or with 15 percent lard or HVO. In contrast, no significant differences in serum levels were observed when the carbohydrate was starch. Okey, Lyman, Harris, and others (*145*) reported that the degree of saturation of dietary fat exerted little influence on serum cholesterol levels when 10 percent of fat was added to a nutritionally adequate synthetic diet. Best, Lucas, Patterson, and Ridout (*23*) also reported that the kind of fat had little effect on serum cholesterol when the diet contained sufficient choline to prevent fatty livers.

Aftergood, Deuel, and Alfin-Slater (*6*) found no significant difference in the plasma cholesterol levels of rats fed a diet containing 15 percent cottonseed oil or lard after a 12-week feeding period. After 24 weeks, however, plasma cholesterol levels were significantly lower in rats fed cottonseed oil than in those receiving lard. Avigan and Steinberg (*15*) reported an increase in serum cholesterol when either coconut oil or corn oil was added to a Purina chow diet, but the increase was somewhat greater with coconut oil than with corn oil.

There is at present no satisfactory explanation for these divergent findings. The results for BHE rats reported in this publication indicate that, in the absence of dietary cholesterol, serum levels change slowly with diet, and suggest that some of the discrepancies in the literature may be due to the relatively short feeding periods generally studied. Another factor that may be responsible for some of the differences observed is the heredity of the strain of rats under investigation. Kohn (*106*) reported evidence for considerable variation among strains of rats in their average serum cholesterol values which varied from 65 to 132 mg./100 ml.

Investigations of the influence of *dietary cholesterol* on the serum cholesterol levels in the rat have dealt chiefly with the addition of cholesterol per se rather than with the use of cholesterol-containing foods. The response to feeding these cholesterol-containing diets is apparently influenced by accompanying dietary components. Dietary cholesterol may be absorbed by the rat in the absence of dietary fat, but the presence of fat in the diet results in an appreciable increase in serum cholesterol levels (*33*). The fatty acid component, not glycerol, is reported to be the important factor (*179*, *189*). Dietary cholesterol may result in elevated values in the blood in the absence of fat if sufficient bile salts are fed (*179*). Wilgram, Lewis, and Best (*189*) reported increased cholesterol levels when choline was added to a diet containing cholesterol.

Unsaturated fats tended to lower serum cholesterol levels of rats fed cholesterol in contrast to the elevated values reported by several investigators when this sterol was absent from the diet. Okey and Stone (*147*) and Aftergood, Deuel, and Alfin-Slater (*6*) reported lower serum values with cottonseed oil than with lard, and small but comparable differences in liver lipids. The latter investigators report that the differences in serum levels observed were not due to differences in the absorption of these two fats. The addition of large amounts of vitamin E to the lard diet eliminated the differences observed in the liver lipid but did not influence blood cholesterol levels.

Nath, Wiener, Harper, and Elvehjem (*136*) demonstrated a marked accumulation of cholesterol in the blood and livers of rats fed 1 percent cholesterol and 10 percent hydrogenated coconut oil, although no accumulation was observed with cholesterol or coconut oil when fed alone. Replacement of 1 percent of the coconut oil with an equivalent amount of corn oil resulted in a marked decrease in blood and liver cholesterol and in a proportionately greater decrease in total liver lipids. Shapiro and Freedman (*170*) found that the addition of safflower oil and methionine to a cholesterol-containing and sulfur-deficient diet was more effective in reducing hypercholesterolemia than a supplement of methionine with a hydrogenated fat (Crisco). No exceedingly high levels of cholesterol were observed with the 13 fats investigated by Okey, Lyman, Harris, and others (*145*) even where cholesterol was included in the diet. The highest value for male rats was 96 mg./100 ml. when coconut oil was fed. Lower values were associated with the more highly unsaturated fats, but there was no consistent trend relating serum levels to the degree of saturation of the dietary fat.

The influence of the unsaturated fats on serum cholesterol levels does not appear to be related to absorption of the sterol. Lin, Karvinen, and Ivy (*117*) and Ivy, Lin, and Karvinen (*99*) reported a limited capacity for cholesterol absorption based on measurements of fecal excretion. Byers and Friedman (*37*) compared the immediate response of rats to cholesterol added to the diet in soybean oil, corn oil, lard, or coconut oil as determined by measurements in intestinal lymph, and found absorption to be greater with the unsaturated than with the saturated fats.

Okey and Lyman (*143*) observed a difference in response to dietary cholesterol depending on the level as well as the kind of dietary fat. Cholesterol levels tended to be higher when cholesterol was fed with 10 percent coconut oil than when fed with an equivalent amount of cottonseed oil. At the 5- and 15-percent levels of these two fats, however, no significant differences were observed.

Very little has been reported on the serum lipids of rats fed cholesterol-containing foods. Blatherwick, Medlar, Bradshaw, and others (*31*) reported high plasma cholesterol as well as fatty livers of high cholesterol content as the result of feeding diets containing large amounts of beef liver. Reussner and Thiessen (*156*) did not determine blood cholesterol values for rats on the cereal and milk or egg and bacon diets, but did obtain evidence of differences in the serum lipid components based on flotation rate measurments that showed a much higher value for the S_f 12–400 class for the bacon and egg diet than for the cereal and milk diet. Rosenkrantz and Bruger (*162*) found that the feeding of egg yolk resulted in an elevation of the cholesterol content in blood and liver.

Although there has been some evidence that dietary cholesterol has little influence on the serum cholesterol of human subjects, evidence has increased indicating that under some circumstances dietary cholesterol may be an important factor in determining serum cholesterol levels.

A single dose of 10 grams of cholesterol fed in a meal with ample fat caused only a small and transient change in the serum cholesterol of young men (*104*). Serum cholesterol levels may be elevated, however, as the result of consuming cholesterol-containing foods such as egg (*34*) or butter (*25*, *26*). From investigations of the response to various fractions from butter with and without various supplements, Beveridge, Connell, Haust, and Mayer (*25*) showed that relatively small amounts of cholesterol, depending on the dietary fat with which it is associated, may effect highly significant increases in plasma cholesterol in man. Beveridge, Connell, Mayer, and Haust (*27*) fed varying levels of cholesterol with a homogenized diet containing 30 percent of the calories as a butter-fat fraction low in cholesterol to a group of university students for a period of 16 days. Between intakes of 13 and 634 mg. of cholesterol daily, serum cholesterol levels increased sharply, but no further significant increases were obtained with daily intakes of 1,300 to 4,500 mg. Cook, Edwards, and Riddell (*43*) reported 15 percent absorption of crystalline cholesterol by one subject (male) in contrast to 60 percent when egg was the source of the sterol. A transient elevation in cholesterol was observed, with serum levels returning to normal within 24 hours. For patients with normal fasting serum cholesterol levels, Messenger, Porosowska, and Steele (*130*) observed an elevation in these levels after feeding egg for a period of 48 days. Okey and Stewart (*146*) and Okey (*141*) demonstrated a slight but consistent rise in the cholesterol level of normal women taking four egg yolks daily for 1 month.

No attempt will be made to review the extensive literature now available on the influence of the degree of saturation of the dietary fat in controlling the level of serum cholesterol in humans. Although many factors complicate interpretation of these studies, such as the short duration of the experimental period, the influence of previous dietary history, and heredity, there is considerable evidence that serum cholesterol levels in humans may be reduced by increasing the proportion of unsaturated fat in the diet (*109*).

The cholesterol level in rats' blood is generally lower than that observed in humans. This relatively low cholesterol level may be due to a species difference or may be a reflection of the lifelong feeding of diets low in fat and cholesterol. The results reported for BHE rats in this publication indicate that elevated cholesterol levels may occur in rats consuming throughout life diets containing relatively high levels of sucrose and higher levels of fat than are usual for this species. The response of the rat to cholesterol-containing diets does not differ markedly from that of humans to comparable diets.

Considerable interest has been evidenced in the plant sterols, such as are present in peanut butter, and their role in lipid metabolism, because of their possible value in reducing blood cholesterol levels. Although there is evidence that plant sterols do result in reduced blood cholesterol under many conditions, the findings on this subject have not been entirely consistent. Here again, the presence or absence of dietary cholesterol seems to be a factor in determining the response to these sterols.

Several recent reports (*76*, *99*, *178*) indicate that plant sterols are absorbed by the rat. Swell, Boiter, Field, and Treadwell (*178*) investigated some of the factors influencing the absorption of these sterols and have suggested that they are absorbed through the same mechanism as cholesterol. A maximum absorption of 22.9 percent was observed when soybean sterols were fed with 25 percent oleic acid and 1 percent sodium taurocholate. Ivy, Lin, and Karvinen (*99*) reported a comparable value for the absorption of soybean sterols and a decrease in absorption of cholesterol when a mixture of the two sterols was fed.

There appears to be little evidence for a decrease in serum cholesterol values when plant sterols are fed to rats on *cholesterol-free* diets. Swell, Boiter, Field, and Treadwell (*178*) obtained an appreciable elevation in the serum level as the result of including 2 percent soybean sterol in a diet containing oleic acid and bile salts. Liver sterols tended to be lowered when plant sterols were included in the diet. Chromatographic analysis provided no evidence of appreciable amounts of plant sterols in either blood or liver, and the rise in the sterol concentration in blood appeared to be due to cholesterol or to a sterol with the same R_f as cholesterol.

When soybean sterols were added to a *diet containing cholesterol*, Swell, Boiter, Field, and Treadwell (*177*) obtained a reduction in blood cholesterol values in comparison with those observed in the absence of the plant sterol. Serum cholesterol levels were found to decrease with increasing concentrations of the plant sterol. Alfin-Slater, Wells, Aftergood, and others (*7*) and Ivy, Lin, and Karvinen (*99*), however, found no appreciable change as the result of adding soybean sterols to a cholesterol-containing diet. The basic diet used by Swell was one that resulted in a marked hypercholesteremia in the absence of plant sterols, whereas the diets used by Alfin-Slater, Wells, Aftergood, and others (*7*), and Ivy, Lin, and Karvinen (*99*) produced blood cholesterol levels only slightly higher than normal.

In view of the results reported for plant sterols fed in the absence of cholesterol, it is possible that phytosterol as well as the unsaturated fat present in peanut butter may be a factor in the elevated serum cholesterol levels reported in this publication for BHE rats fed SPPB diet.

The kind and level of dietary protein (*61*, *134*, *135*, *142*, *144*, *147*) and the type of dietary carbohydrate (*3*, *82*, *103*, *152*, *153*) have been implicated as factors of importance in determining serum cholesterol levels in the rat. The investigations reported in this publication were not planned to determine the role of either of these dietary components.

The level of protein was relatively constant in the diets of BHE rats except for the diets consisting of 100 percent whole egg or egg yolk. The data available were insufficient to determine whether the high level of protein in Y100 diet was a factor in lowering the serum cholesterol of these rats. The possible effect of specific proteins in combination with other dietary ingredients as factors influencing serum cholesterol levels has not been excluded.

Sucrose was the dietary carbohydrate for most of the diets fed to BHE rats. The only diets without high levels of sucrose were the stock diet, E100, and Y100 diets. Differences in the response of rats to diets containing 25 percent whole egg (SPE) and those containing 100 percent egg (E100) or 100 percent egg yolk (Y100) may be related to the lack of sucrose in these last two diets.

The thyroid gland has long been recognized as exerting appreciable influence on lipid metabolism and is the most important of the endocrine glands as regards the control of cholesterol metabolism. Handler (*84*) reported a marked increase in the cholesterol concentration of the liver and serum of the rat in the hypothyroid state. Thyroid feeding resulted in a decrease in the cholesterol concentration of the liver and effected a relatively small decrease in serum levels. Although cholesterol synthesis and absorption have been found to increase in the hyperthyroid rat, it appears that the excretory or destructive processes concerned with cholesterol dominate in the hyperthyroid state (*36*).

However, as the result of injecting anti-rat kidney serum (AKS) into rats previously fed with thyroid to produce hyperthyroidism, Rosenman and Smith (*165*) reported a marked increase in plasma cholesterol. Under these conditions the hypothyroid rat showed a lowering of the plasma cholesterol when compared with the control animal. It appears that this effect on the hyperthyroid rat was due to a metabolic block to lipid egress resulting from the anti-rat kidney serum which permitted the accumulation of cholesterol in the blood and not to any change in the hyperthyroid state.

The enlarged thyroids here reported for BHE rats fed SPE and SPPB diets were generally accompanied by elevated serum cholesterol levels. There was no evidence microscopically of any abnormality in the thyroids of the rats that were maintaining weight, and no data were available to determine the possible influence of these diets on the excretion of this sterol.

There has been considerable evidence associating hypercholesterolemia with nephrosis in man and in animals. Hyperlipemia has also been observed

to accompany the nephrotic state. Moribund BHE rats, regardless of diet, frequently exhibited a hypercholesterolemia generally associated with damaged kidneys. Blood sera from these rats were often obviously lipemic. In many ways the picture seen in these moribund rats was similar to that observed in experimentally induced nephrotic rats.

Heymann and Lund (*90*) showed that a condition simulating the nephrotic syndrome of childhood can be produced in rats by the injection of rabbit anti-rat kidney serum (AKS) prepared by immunizing rabbits against rat kidney. This procedure has been used rather extensively to study the factors involved in chronic nephrosis in this animal. Heymann, Matthews, Lemm, and others (*91*) observed no evidence of a disturbed clearance of fat from blood when intravenous injections of C^{14} labeled trilaurin were given to nephrotic rats. Rosenman, Friedman, and Byers (*163*) reported that the hypercholesterolemia observed was not due to increased intestinal absorption, to decreased rate of excretion, nor to increased cholesterol synthesis. The elevated blood cholesterol present in these rats was endogenous in origin, and the authors have suggested biliary obstruction as the cause. Friedman, Rosenman, and Byers (*71*) found that the nephrotic rat was unable to remove either endogenously or exogenously derived lipid from plasma with its usual efficiency. A progressive fall in plasma albumin was found to follow the injection of AKS and was associated with a rise in plasma triglycerides, phospholipids, and total cholesterol. Heymann and Hackel (*88, 89*) indicated a possible involvement of both the kidney and liver in the mechanism eliciting hyperlipemia. Bilateral nephrectomy (*88*) prevented the development of hyperlipemia, and it was suggested that a "hyperlipemia inducing" agent may be secreted by the nephrotic kidney. Subtotal hepatectomy (*89*) resulted in reduced hyperlipemia. Ehrich, Forman, and Seifer (*55*) reported an increased kidney weight, an increased adrenal weight, and extensive proteinuria in rats receiving a large dosage of AKS.

Lewis and Heymann (*115*) analyzed the serum lipoproteins in these rats and found the greatest increment in the low density fractions. They were similar in type to those of nephrotic children. Heymann, Matthews, Lemm, and others (*91*) suggested that the hyperlipemia observed was due to increased mobilization of lipid rather than to a deposit of lipid in tissues. Marsh and Drabkin (*124*) provided evidence indicating that fat was mobilized from body stores.

There appears to be little information on the production by dietary means of hypercholesterolemia and hyperlipemia in rats associated with kidney damage. According to Blatherwick and Medlar (*30*), diet alone will produce nephritis and will also determine its severity. They observed marked involvement of the kidney when rats were fed a diet containing 75 percent liver. Some kidney damage was also found when the level of liver fed was 30 percent. Fatty infiltration of the liver, high liver and plasma cholesterol, and increased urinary protein were observed. An average plasma cholesterol of 88 mg./100 ml. was observed in stock rats without nephritis. Values over 146 mg./100 ml. were considered hypercholesteremic. On liver diet, the mean value was 126 mg./100 ml. without nephritis and 219 mg./100 ml. was considered the upper level for normal rats. Higher values were associated with extensive kidney damage. Fatty infiltration of the liver was observed even though kidneys still appeared normal in rats fed the high level of liver.

The fatty infiltration of the liver, the high plasma cholesterol, and the increased urinary protein obtained by Blatherwick and Medlar (*30*) when rats were fed a diet containing high levels of liver were strikingly similar to the results reported here for BHE rats fed diets containing 25 percent egg.

Summary.—The results of the investigations reported in this bulletin provide further evidence that many factors influence blood cholesterol levels, and emphasize the statement made by Portman and Stare (*153*) that it is unwise to place too much emphasis on the effect of a single factor in the control of serum cholesterol unless that factor is evaluated under a wide range of conditions.

From the long-term studies with BHE rats, the relation of serum cholesterol levels to age was found to differ with diet. High levels were rarely seen in healthy BHE rats that were maintaining their weight on the stock diet and there was little evidence that cholesterol levels were influenced by age. Cholesterol levels were generally low in rats under 400 days old that were maintaining their weight on the semipurified diet or on modifications of this diet, SPM, SPB, and SPPB, containing milk, beef, or peanut butter. In the sera of older rats, elevated cholesterol levels were observed on all of these diets except the stock diet; the highest value observed in the absence of dietary cholesterol was for rats fed SPPB diet. The increased serum cholesterol in the older rats was generally accompanied by kidneys showing evidence of degenerative changes even in rats that appeared healthy at the time of sacrifice.

Although serum cholesterol values tended to be high in moribund rats, relatively low cholesterol levels were found in several rats that survived over 700 days. Cholesterol values obtained for individual rats at intervals throughout life are needed to determine whether we are measuring changes in cholesterol level that are due to aging processes or whether these changes may be the result of the development of some pathological condition.

In the presence of dietary cholesterol, with egg as the source, elevated cholesterol values were observed in relatively young BHE rats. The values for rats between 200 and 400 days

of age were significantly higher than those observed on the other experimental diets. Exceedingly high values were observed for rats 300 to 400 days of age. Elevated serum cholesterol values were seen before there was evidence of kidney damage, and showed no relation to the amount of liver fat. The addition of cholesterol to the SPE diet already high in cholesterol appeared to exert little influence on the serum level of this sterol, indicating that a saturation level had been reached with the diet containing 25 percent egg. Dietary cholesterol alone was not responsible for the high serum cholesterol levels in SPE rats; the values for rats fed the exceedingly high cholesterol-containing diet Y100 were significantly lower than those with SPE diet. In the levels fed, there was no evidence that supplementation with choline, vitamin B_6, vitamin B_{12}, or ascorbic acid exerted a significant influence on the cholesterol levels found in moribund rats fed SPE diet. A serum cholesterol level of 160 mg./100 ml. appeared to be the upper limit for serum levels associated with normal kidneys in stock or SP 8 HVO rats; the upper limit for rats fed SPE diet was 250 mg./100 ml.

Serum protein components

Rats maintaining weight on stock, SP 8 HVO, and SPE diets.—In table 71 are summarized data from electrophoretic analysis of blood serum from rats maintaining weight on stock, SP 8 HVO, and SPE diets. The data reported are for fasted rats. The results are reported as percentage of total protein; no data were available for total serum protein. Values for albumin and $alpha_1$ globulin have been combined because of difficulty encountered in obtaining a clear-cut separation of these components for some of the serum samples analyzed. The presence of one or more components with an electrophoretic mobility faster than that of albumin (PA) was of particular interest. Because of the influence of this prealbumin component on the relative percentage of the other serum proteins, data for serum samples with no PA present are reported separately from those with PA.

The usual serum protein components with a normal distribution were found in the serum of rats less than 300 days old that were maintaining their weight on the *stock diet*. In rats over 300 days of age, serum proteins contained smaller concentrations of albumin and $alpha_1$ globulin and higher concentrations of the other components than those in the younger rats. In some rats small amounts of a fast-moving component were found, generally represented by a diffuse band rather than by a clear-cut peak or peaks. The highest value observed for this fast-moving component was 2.4 percent of the total serum proteins.

The results with SP 8 HVO diet were in general similar to those with stock diet except for the greater proportion of rats with PA in their sera. This component was present in the sera from some of the rats that were less than 300 days old, and was found in 67 percent of the animals more than 300 days old. Some relatively high concentrations of this component were found in sera from the oldest group of rats. The relative concentration of $alpha_2$ globulin also tended to increase with age.

On *SPE diet,* the fast-moving component was present at all ages, generally showing a more distinct separation, and evidence of more than one fast-moving component was frequently obtained. Between 300 and 400 days, the proportion of animals with sera containing PA was large and the amounts of PA tended to be high. This component was absent from all but one of the sera from rats 400 to 499 days old but again was seen in a high percentage of the rats 500 days old. The significance of this decrease in PA in the 400- to 499-day-old group was not apparent but may be related to the ability of these rats to survive the critical 300- to 399-day period. The relative concentration of the other protein components varied and showed no consistent trend with age. The concentration of gamma globulin in the sera of SPE rats tended to be low whether or not PA was present.

Rats losing weight on stock, SP 8 HVO, and SPE diets.—In table 72 are summarized data for rats that were losing weight on these same diets. The data available for moribund or sick rats fed stock diet were limited in number and were for older rats. Except for a higher concentration of PA, the results were similar to those obtained for rats of comparable age that were maintaining weight on this diet.

The relative concentration of albumin and $alpha_1$ globulin tended to be lower in rats that were losing weight than in those maintaining weight on *SP 8 HVO diet,* and difficulty was encountered, in the older rats, in obtaining a separation of these components from $alpha_2$ globulin. PA was absent from the sera of rats under 300 days old and was low when present in the sera of rats over 600 days of age. Of interest was the exceedingly high PA value of 41.8 percent in one rat with a liver tumor. This value has been excluded from the results in table 72.

On SPE diet, large amounts of PA were found in the sera of moribund rats of all age groups 300 days and over, including the 400- to 499-day-old animals. There were too few data to assess accurately the influence of the extent of weight loss on the serum proteins, but it may be of significance that the three rats over 300 days old showing no PA had all lost over 100 grams.

Serum cholesterol, kidney size, and damage in relation to PA.—In table 73 are summarized data on cholesterol levels and kidney size and damage as related to increasing amounts of the fast-moving component in the sera of rats fed SP 8 HVO and SPE diets. On *SP 8 HVO diet,* when

TABLE 71.—*Protein components of sera from rats maintaining weight at different ages on stock, SP 8 HVO, and SPE diets*

Diet and age of rats (days)	Rats	Average age	Composition of serum proteins, rats without PA[1]				Rats	Average age	Composition of serum proteins, rats with PA[1]					
			Albumin + $alpha_1$ globulin	$Alpha_2$ globulin	Beta globulin	Gamma globulin			PA[1] Average	PA[1] Range	Albumin + $alpha_1$ globulin	$Alpha_2$ globulin	Beta globulin	Gamma globulin
	Number	*Days*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Days*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Stock:														
Less than 300	7	192	70. 3	6. 5	15. 9	7. 3	-----	-----	-----	-----	-----	-----	-----	-----
300 to 499	4	420	54. 9	8. 1	21. 6	15. 4	1	373	1. 1	1. 1	62. 7	7. 9	20. 7	7. 5
500 and over	2	764	58. 0	7. 0	22. 1	12. 9	3	792	1. 6	1. 1– 2. 4	61. 1	9. 2	17. 5	10. 7
SP 8 HVO:														
Less than 300	4	250	68. 6	5. 8	17. 3	8. 2	2	255	3. 0	2. 9, 3. 2	74. 4	4. 2	15. 6	2. 8
300 to 399	6	353	64. 2	8. 4	17. 0	10. 3	2	352	3. 3	2. 8, 3. 8	63. 2	7. 3	16. 2	10. 0
400 to 499	3	453	67. 3	8. 0	16. 3	8. 4	3	456	3. 8	2. 2– 6. 2	64. 1	9. 3	15. 2	7. 6
500 to 599	3	548	54. 4	11. 2	21. 6	12. 7	6	546	8. 0	3. 9–17. 6	57. 1	10. 0	16. 4	8. 1
SPE:														
Less than 300	5	251	70. 4	7. 7	14. 8	7. 1	2	253	4. 9	2. 5, 7. 4	65. 8	7. 3	15. 4	6. 6
300 to 399	2	358	73. 4	7. 2	14. 3	5. 1	5	351	9. 5	4. 1–19. 3	67. 3	6. 7	12. 1	4. 5
400 to 499	6	455	70. 9	8. 0	15. 9	5. 1	1	494	5. 3	5. 3	65. 2	7. 5	17. 2	4. 8
500 to 599	4	532	61. 7	11. 2	19. 0	8. 0	5	520	6. 1	3. 7–13. 8	63. 6	9. 1	14. 5	6. 8

[1] PA represents prealbumin component or components moving more rapidly than albumin.

TABLE 72.—*Protein components of sera from rats losing weight at different ages on stock, SP 8 HVO, and SPE diets*

Diet and age of rats (days)	Rats	Average age	Composition of serum proteins, rats without PA[1]: Albumin + alpha$_1$ globulin	Alpha$_2$ globulin	Beta globulin	Gamma globulin	Rats	Average age	Composition of serum proteins, rats with PA[1]: PA[1] Average	PA[1] Range	Albumin + alpha$_1$ globulin	Alpha$_2$ globulin	Beta globulin	Gamma globulin
	Number	*Days*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Days*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Stock:														
500 and over	1	684	61.1	7.8	21.5	9.6	3	672	5.5	3.2–8.1	60.1	7.5	15.6	11.0
SP 8 HVO:														
Less than 400	3	317	56.9	11.7	19.9	11.4								
400 to 599	3	484	55.3	8.5	24.6	11.6	1	483	7.9	7.9	50.0	11.5	21.7	9.0
600 and over	3	710	[2]62.3		20.5	17.2	3	753	1.7	1.2–2.6	[2]66.0		22.0	10.3
SPE:														
Less than 300	1	281	75.0	7.3	12.0	5.6								
300 to 399	1	345	64.8	15.4	16.4	3.4	8	363	7.0	2.7–13.4	67.4	8.0	12.8	4.9
400 to 499	1	474	74.0	8.0	12.1	5.9	8	440	5.7	0.8–10.3	62.9	8.9	15.6	7.0
500 to 599	1	513	56.2	8.9	21.4	13.5	6	529	8.8	2.8–12.0	65.2	9.4	12.2	4.4

[1] PA represents prealbumin component or components moving more rapidly than albumin.
[2] Poor separation: Value represents combined albumin and alpha$_1$ and alpha$_2$ globulins.

TABLE 73.—*PA,[1] serum cholesterol, kidney weight, and kind and extent of kidney damage in rats fed SP 8 HVO and SPE diets*

Diet and PA range	Rats	Average age	Average PA [2]	Serum cholesterol [2]		Kidney weight		Rating of kidney damage		
				Average	Range	Average	Range	Hyalin	Cystic	Glomerular
SP 8 HVO:	*Number*	*Days*	*Percent*	*Mg./100 ml.*	*Mg./100 ml.*	*Grams*	*Grams*	*Score*	*Score*	*Score*
PA, none	25	426	0	117	81–185	1. 7	1. 2– 3. 4	0. 3	0	0
Less than 4.0	10	493	2. 7	129	112–192	1. 5	1. 2– 2. 0	. 3	0	0
4.0 to 7.9	6	520	6. 8	188	108–278	2. 5	1. 8– 4. 5	1. 5	0. 5	0. 5
8.0 and over	1	544	17. 6	270	270	5. 2	5. 2	2. 0	3. 0	2. 0
SPE:										
PA, none	20	405	0	225	152–326	[3] 2. 2	1. 5– 4. 7	1. 2	. 4	. 2
Less than 4.0	8	419	2. 6	286	132–434	2. 1	1. 8– 3. 6	1. 2	0	0
4.0 to 7.9	15	426	5. 7	314	152–437	4. 8	1. 6–10. 1	1. 8	1. 5	. 9
8.0 and over	12	434	11. 7	488	237–743	6. 1	1. 8–11. 2	2. 1	2. 7	1. 2

[1] PA represents prealbumin component or components moving more rapidly than albumin.
[2] PA values represent relative percentage of serum protein components. Cholesterol values represent milligrams per 100 milliliters in serum.
[3] Omitting one rat with kidney weight of 13.2 grams—no PA and cholesterol 202 mg./100 ml.

PA was absent from the sera, cholesterol levels were generally low and the kidney small with little evidence of damage. Only 3 of the 25 rats in this group had kidneys exceeding 2.0 grams in weight, and only 5 had serum cholesterol levels exceeding 150 mg./100 ml. When the PA level in the serum was less than 4 percent of the serum proteins, cholesterol levels and kidney weights were similar to those for rats with no PA in their sera. When the PA level was above 4.0 percent, kidneys were generally enlarged and cholesterol levels exceeded 150 mg./100 ml. in all but 1 rat.

On *SPE diet* a similar trend was observed, although the range of values was wide for each group. In the group of 20 rats with no PA in their serum protein, only 1 had a serum cholesterol level in excess of 300 mg./100 ml. and 2 of the kidneys exceeded 3.0 grams in weight. Excluded from the data was 1 rat with a very large kidney weighing 13.2 grams. This rat had no PA in the serum and a serum cholesterol of 202 mg./100 ml. In the group of 12 rats with PA levels 8 percent and over, only 1 had a serum cholesterol value of less than 300 mg./100 ml.; the others had values exceeding 400 mg./100 ml. Three of the kidneys weighed less than 3 grams; eight exceeded 6.0 grams. In general, high concentrations of PA in serum proteins tended to parallel serum cholesterol levels somewhat more closely than kidney size.

RATS FED SPM, SPB, AND SPPB DIETS.—In table 74 are summarized limited data from electrophoresis of the sera from rats fed SPM, SPB, and SPPB diets. There were insufficient data to establish the influence of age. With each of these diets the fast-moving component was present in the sera of some of the rats, even among the relatively young animals. The highest level observed was for a rat fed SPM diet, with the fast-moving component representing 20 percent of the serum proteins. The other serum proteins were present in amounts that were similar to those found in rats fed SP 8 HVO or stock diets.

DISCUSSION.—Many factors have been shown to influence the results of electrophoretic studies of blood proteins, and hence to complicate comparisons of the results of such studies (*28, 49, 133*). Concentration and kind of buffer, optical devices used to resolve the protein concentration gradients, and species, strain, sex, and age of the experimental animals may all be determining factors. Many of the investigations have dealt with attempts to characterize certain pathological conditions by means of the electrophoretic pattern of the blood proteins. There appear to be no reports of investigations comparable to those included in this bulletin dealing with the electrophoretic pattern of the blood proteins of rats on various dietary regimens throughout their lifespan.

In 1945, Deutsch and Goodloe (*49*) investigated the plasma proteins of 20 species of animals and obtained evidence of a small amount of protein migrating more rapidly than albumin in certain species, including the rat. These authors reported poor electrophoretic separation for some of the proteins in the blood plasma of rats. Halliday and Kekwick (*83*) reported, in the blood of young rats, a component moving ahead of albumin, possibly a second albumin, which varied in concentration from 8.6 percent at 12 days of age to 4.2 percent at 90 days. Total albumin increased from 60 to 70 percent of the total protein during this period. A preliminary report (*38*) from this laboratory indicated the presence of high levels of rapidly migrating proteins in the blood serum of rats receiving a diet containing 25 percent cooked dried whole egg.

Although data on rapidly moving components in the sera of rats are limited, considerable attention has been given to their occurrence in the sera of humans, variously designated as PA (prealbu-

TABLE 74.—*Protein components of sera from rats at different ages on SPM, SPB, and SPPB diets*

Weight status, diet, and age of rats (days)	Rats	Average age	Composition of serum proteins, rats without PA[1]: Albumin + alpha₁ globulin	Alpha₂ globulin	Beta globulin	Gamma globulin	Rats	Average age	Composition of serum proteins, rats with PA[1]: PA[1] Average	PA[1] Range	Albumin + alpha₁ globulin	Alpha₂ globulin	Beta globulin	Gamma globulin
	Number	*Days*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Days*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Maintaining weight:														
SPM:														
Less than 400	2	300	66. 1	6. 9	17. 2	9. 8	4	306	4. 1	2. 4– 6. 4	67. 9	5. 6	15. 6	6. 8
400 to 599	2	444	64. 4	6. 2	18. 8	10. 7	2	543	5. 2	4. 8, 5. 5	63. 3	11. 5	[2] 20. 1	
SPB:														
Less than 400	3	290	63. 1	7. 0	16. 9	13. 1	3	319	3. 7	1. 9– 5. 2	66. 6	7. 1	15. 4	7. 2
400 to 599	2	488	53. 6	10. 6	21. 1	14. 7	1	453	2. 9	2. 9	69. 3	6. 4	14. 5	6. 9
SPPB:														
Less than 400	3	291	65. 0	7. 2	18. 4	9. 4	2	302	2. 6	0. 7, 4. 6	64. 0	6. 3	18. 5	8. 5
400 to 599	------	------	------	------	------	------	4	519	6. 2	3. 8– 7. 5	64. 6	8. 0	14. 0	7. 2
Losing weight:														
SPM	2	327	58. 0	4. 7	25. 5	11. 9	3	545	11. 4	6. 4–20. 1	[3] 67. 7		15. 9	5. 1
SPB	2	546	67. 2	6. 7	18. 7	7. 4	3	598	6. 6	1. 0–15. 0	[3] 66. 4		20. 4	6. 7
SPPB	2	407	[3] 70. 2		18. 2	11. 6	3	344	5. 7	3. 3– 8. 4	[3] 73. 1		15. 6	5. 6

[1] PA represents prealbumin component or components moving more rapidly than albumin.
[2] Poor separation: Value represents combined beta and gamma globulins.
[3] Poor separation: Value represents combined albumin and alpha₁ and alpha₂ globulins.

min), FC (fast moving) or Rho (rapid). Azerod, Lewin, and Ghata (*16*) obtained evidence of two prealbumin protein fractions in normal human serum, an electrophoretically homogeneous fraction migrating slightly faster than albumin and a heterogeneous fraction spread over a large area in front of the albumin. A change in the mobility of the blood proteins and the presence of a rapidly moving prealbumin fraction as the result of the administration of heparin to lipemic individuals was noted by Nikkilä (*139*), Lever, Smith, and Hurley (*113, 114*), and Herbst and Hurley (*87*).

The influence of heparin on the blood proteins appears to be attributable to the liberation of a lipoprotein lipase (*107*) into the blood and to the production of more rapidly migrating fractions due to the association of the fatty acids with some of the blood proteins (*75*). Interest in this heparin clearing reaction has been evidenced because of its possible role in fat transport. The concentration of this clearing factor in blood serum is normally low, and the physiological importance of the reaction has not been definitely established. Many of the investigations have dealt with measurements of enzyme activity as determined by clearing of lipemic sera or liberation of free fatty acids, and have not included electrophoretic measurements of the blood proteins. The present status of our knowledge of clearing factor has been reviewed by Robinson (*159*) and Engelberg (*57*).

The data available from electrophoretic studies of sera from BHE rats have provided no information concerning the chemical nature of these fast-moving components and no proof that these components were the same as those resulting from the administration of heparin. There seemed to be considerable indirect evidence linking high level of this component to the lipid metabolism of these rats. Elevated serum cholesterol values were obtained for the majority of the rats with high levels of PA in their sera. The lipemic sera that were encountered frequently, even after the usual 17-hour fast, were associated generally with high levels of one or more fast-moving components. Rosenman and Smith (*164*) indicated a possible causal relationship between deficiency of albumin and increased lipid content of nephrotic plasma. Whether or not the albumin content of the blood of BHE rats was a factor in the results obtained could not be determined from the data available. The relative values for the various protein components in serum protein provide no information on the actual concentration of these fractions in the serum. Further investigations are underway to determine the possible physiological significance of PA and the relation of these components to lipid metabolism.

Summary.—With the BHE strain of rats, age and diet were found to influence the relative amount of the various protein components. Of particular interest was the frequent occurrence of a component or components moving more rapidly than albumin (PA).

In stock rats 300 days or younger, there was no evidence of the fast-moving component and, except in the sera of a few moribund rats, the amounts of PA were relatively small even in the older animals. PA was present in the sera of some of the younger rats fed the semipurified diet or SPM, SPB, or SPPB diets, with a tendency for high levels in the older rats. On SPE diet, levels of PA tended to be high at all ages, with the largest proportion of rats with serum containing this component in the 300- to 399-day-old group.

Small amounts of PA were observed in approximately 50 percent of the rats with normal kidneys and serum cholesterol levels, irrespective of the experimental diet. When serum levels of PA were high, they were often associated with enlarged and damaged kidneys and with high cholesterol levels.

General Summary and Implications for Future Research

Results are reported from long-term studies with male rats dealing with the influence of diet on length of life and changes that occur with age in blood serum and in livers, kidneys, adrenals, and thyroids. The diets investigated were modifications of a relatively simple semipurified diet. Egg, beef, milk, or peanut butter were substituted for 20 to 25 percent of the semipurified diet in one series of experimental diets; the kind and level of fat in the semipurified diet was varied in the second series of diets. The fats included a hydrogenated vegetable oil, lard, and butter, and the levels used were 8 and 16 percent. For comparative purposes, data were obtained for animals raised on the diet routinely used for maintaining the laboratory stock colony. Results showing the influence of fasting and of weight loss before sacrifice have also been included. Most of the data reported are for BHE rats, a mixed strain of animals bred in our stock colony, but also included are the results of feeding a group of Wistar rats the semipurified diet and the diet containing 25 percent cooked dried egg.

The animals grew well on all of the experimental diets and generally attained a maximum weight greater than that observed with rats on the stock diet. During the period of early growth, rats fed the diets containing egg, milk, beef, or peanut

butter grew more rapidly and used their diet more efficiently for growth than did those fed the semipurified diet. Many of the rats continued to gain in weight as long as they remained healthy and some became extremely large, particularly when the diets contained milk or peanut butter. The differences in body weight observed were not consistently related to food intake or to level of dietary fat.

The lifespan of BHE rats fed the various experimental diets differed widely. At death, kidney damage was a frequent finding regardless of diet. The extent of the damage varied among diets and generally paralleled the length of survival. The shortest average lifespan and the most extensive kidney damage were observed with diets containing 25 percent egg. When rats fed the egg-containing diet, SPE, during the first 250 days of life were then placed on stock ration, their lifespan was significantly longer than when the SPE diet was fed throughout life. When the feeding of this diet was delayed until the rats reached 250 days of age, length of life was also prolonged. Survival was longer with a diet consisting of 100 percent whole egg than with one containing 25 percent egg. Some extremely long-lived rats were obtained when the milk-containing diet was fed. When the dietary fat was HVO, animals tended to live longer than when the fat was lard or butter.

No evidence was obtained of any nutritional deficiency in the diets under investigation. Neither level of fat nor level of protein explained the differences observed. The results, particularly those with 100 percent whole egg or egg yolk in contrast to those with 25 percent egg, suggest that the rate of the development of untoward changes in the tissues was related to the particular combination of nutrients under investigation rather than to any one dietary ingredient.

Research is needed to determine the specific combination of nutrients responsible for accelerating changes in the tissues which result in early death, and to establish whether or not the age span during which the diets are fed is a critical factor in determining the response to such diets. The possibility also should be explored of establishing criteria that would detect at an early age possible adverse effects of specific nutrient combinations.

A factor that appears to complicate interpretation of the longevity data is the tendency for some rats to eat excessively and to gain at a very rapid rate. Animals weighing 600 grams or more by the time they reached 200 days of age died at an early age regardless of diet.

Comparative studies of rats fed controlled as well as ad libitum amounts of food are needed to permit a more accurate assessment of the data on longevity in relation to diet, as well as to determine the possible adverse effect of excessive food consumption at various stages in the life cycle.

Microscopic examination of the tissues indicated that the kidney was the organ most frequently found to appear abnormal, and that kidney damage was observed in rats showing no obvious signs of ill health, as well as in moribund rats. Some diets obviously hastened the onset of lesions and also appeared to influence the type of degenerative changes observed. A kidney weighing more than 1.8 grams usually showed evidence of degenerative change, and extensive damage was apparent in kidneys weighing more than 3 grams. The influence of diet on the composition of the kidney depended chiefly on its influence on the size of this organ. Enlarged kidneys generally contained a relatively high percentage of protein and a low percentage of fat. Although high ash values were also found frequently, calcium deposition as determined microscopically did not necessarily parallel the percentage of ash in the kidney.

Microscopic examination of the livers revealed little evidence of degenerative changes in this organ, although both histological and chemical measurements showed a high fat content in the livers of rats on diets containing high levels of egg.

The kidneys as well as the livers from rats subjected to a 17-hour fast before sacrifice were generally smaller than those from nonfasted rats. The increasing difference with age between the weight of these organs from fasted and nonfasted rats fed stock diet suggests that the physiological activity of these organs is decreasing with age. Calcium deposits in the kidney also appeared to depend upon the fasting state of the rat at the time of sacrifice.

Comparative data on the tissues of fasted and nonfasted rats at different intervals throughout life might well contribute information on the aging processes.

The size of the adrenal and thyroid glands seemed to be influenced by diet when comparisons were made for animals that were maintaining weight when sacrificed. The influence of diet on the thyroid weight was apparent even in relatively young rats, whereas the influence of diet on the adrenal was seen chiefly in older rats. In moribund rats, large adrenals and thyroids were a frequent finding regardless of diet.

In rats that were maintaining weight at the time of sacrifice, serum cholesterol levels varied with age and with diet and appeared to bear no consistent relation to level of dietary cholesterol or fat. With the stock diet, serum levels were generally low at all ages. With the various modifications of the semipurified diet, serum cholesterol levels tended to be high in older rats. Some extremely high levels were observed, even at a relatively early age, in rats fed the cholesterol-containing egg diets. Cholesterol levels also tended to be high in rats fed the diet containing 20 percent peanut butter, although the cholesterol content of this diet was low. In moribund rats, serum cholesterol levels exceeding 200 mg./100 ml. were observed frequently regardless of diet,

and generally were accompanied by enlarged and damaged kidneys.

A rapidly moving protein component in the blood serum of rats was observed occasionally at all ages and on all diets. The percentage of rats with this component in their sera, as well as the amount present, varied with age and with diet. The presence of small amounts of PA observed occasionally in young rats seemed to bear little relation to diet. When high levels of this component were observed, they were usually associated with extensively damaged kidneys and elevated serum cholesterol levels.

Histological and biochemical investigations of the tissues of moribund rats measure only terminal stages and provide little information concerning the intermediate steps leading to death and the effect of diet thereon.

More extensive studies dealing with the changes that occur at different stages of the life cycle are needed to establish the role of diet in the sequence of events that determine length of life.

The limited data for Wistar rats fed the semipurified diet and the diet containing 25 percent egg indicate that the response to diet may differ markedly with the strain of rats under investigation. Wistar rats lived longer than BHE rats fed both diets, with greater differences observed when the egg-containing diet was fed. Even in older rats of the Wistar strain, kidney damage was rarely seen and appeared to be unrelated to the diet.

To explain such differences in the response to diet, comparative data for different strains of animals should include measurements to detect basic differences in tissue enzymes and in the metabolic pathways controlling the utilization of various experimental diets. The possibility of detecting inherent differences at an early age and of controlling or preventing by dietary means the adverse effects due to heredity also warrants further consideration.

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Appendix

The tables in the appendix supply additional data not included in the text.

In tables 75 through 78 are summarized the data for the ingredients used in making the experimental diets. These tables serve as a basis for the calculated values recorded in tables 2 through 6.

In tables 79 and 80 are presented data on weight and intake of individual series of rats, showing the general agreement among different experimenta· series of animals when fed the same diet.

In table 81 are presented data for individual animals when a change in dietary regimen was made at 250 days of age. Although the data for this group of rats were extremely limited, the individual data show that consistent differences were observed for all of the rats as the result of this change.

TABLE 75.—*Protein and amino acid composition per 100 grams of ingredients in experimental diets* [1]

Food	Nitrogen [2]	Protein factor	Protein	Tryptophane	Threonine	Isoleucine	Leucine	Lysine	Methionine	Cystine	Phenylalanine	Tyrosine	Valine	Arginine	Histidine	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine
	Grams		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Casein	13.83	6.29	87.0	1.16	3.72	5.70	8.74	6.97	2.68	0.33	4.69	5.06	6.43	3.54	2.63	2.92	6.43	20.05	1.73	10.22	5.78
Lactalbumin	12.31	6.49	79.9	1.76	4.19	4.96	9.86	7.24	1.80	2.72	3.48	3.04	4.54	2.79	1.53	5.07	7.92	12.85	------	3.30	3.30
Yeast	8.34	6.25	52.1	.80	2.65	2.70	3.64	3.72	.94	.62	2.14	2.14	3.07	2.54	1.41	3.89	5.90	7.14	2.74	2.24	2.99
Dried egg	7.49	6.25	46.8	.77	2.33	3.11	4.12	3.00	1.47	1.09	2.70	2.01	3.48	3.07	1.12	------	3.28	5.79	1.66	1.98	3.93
Dried egg yolk	4.73	6.25	29.5	.43	1.50	1.81	2.49	1.95	.76	.50	1.30	1.37	2.03	2.05	.67	------	1.63	3.54	1.04	1.31	2.62
Dried egg white	12.91	6.25	80.7	1.23	3.56	5.22	7.10	4.84	3.14	1.96	5.15	3.36	6.29	4.74	1.74	6.09	6.35	11.36	3.02	3.05	6.34
Skim milk solids	5.57	6.38	35.6	.50	1.64	2.27	3.49	2.76	.87	.32	1.72	1.81	2.44	1.30	.94	1.23	2.59	8.30	.70	3.95	2.10
Beef	12.21	6.25	76.3	.89	3.37	3.99	6.25	6.67	1.89	.96	3.14	2.59	4.24	4.92	2.65	4.41	7.12	11.55	4.72	3.76	3.20
Peanut butter	4.84	5.46	26.4	.33	.81	1.24	1.84	1.08	.27	.46	1.53	1.08	1.50	3.24	.74	1.07	4.25	5.83	1.68	1.54	1.99

[1] Source of values for nitrogen and protein factor from which protein was calculated, (*101*), (*129*, p. 4); all others, (*148*).
[2] Analyzed values.

Table 76.—*Lipid composition per 100 grams of crude fat and of ingredients in experimental diets*

Item	Total fat [1]	Saturated fatty acids										Unsaturated fatty acids						Cholesterol	Iodine value
		Total	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	Total	Mono- C_{10}–C_{16}	Mono- C_{18}	Di- C_{18}	Tri- C_{18}	All C_{20}–C_{22}		
Fats	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Mg.*	
HVO [2a]	100	22.6						0.6	14.1	6.3	1.6	73.0	0.3	65.4	7.2	0.2			74.6
Lard [2b]	100	38.2							30.8	7.5		57.4		45.9	10.5	.6	0.4	[3] 114	[2b] 64.0
Butterfat [2c]	100	56.2	3.1	1.7	0.8	1.3	3.6	7.9	25.8	12.0		39.4	2.0	33.5	2.9	.8	.3	[3] 235	[2c] 33.4
Egg fat [2d]	100	30.5						.4	24.2	5.9		65.1	5.5	47.8	7.8	1.4	2.5		[4] 84
Beef suet [4]	100	48.0							28	19		47		44	2				[4] 47
Peanut oil [2e]	100	25.7						1.1	11.3	5.7	7.6	69.9		36.3	33.6			[5] 108	[4] 100
Ingredients																			
Casein [6]	1.51	0.85	0.05	0.03	0.01	0.02	0.06	0.12	0.39	0.18		0.59	0.03	0.50	0.04	0.01	Trace	[3] 65	
Lactalbumin [6]	7.92	4.45	.24	.14	.06	.11	.29	.63	2.04	.95		3.12	.16	2.65	.23	.06	0.02		
Whole dried egg [7]	42.7	13.02						.17	10.3	2.52		27.8	2.35	20.4	3.33	.60	1.07	[1] 1,880	
Dried egg yolk [7]	58.0	17.69						.23	14.0	.34		37.8	3.19	27.7	4.52	.81	1.45	[3] 2,780	
Skim milk solids [6]	1.28	.72	.04	.02	.01	.02	.05	.10	.33	.15		.50	.03	.43	.04	.01	Trace	[8] 30	
Beef [7]	14.8	6.96							4.14	2.81		6.81		6.51	.30			[5] 157	
Peanut butter [7]	50.3	12.93						.55	5.68	2.87	3.82	35.16		18.26	16.9			0	

[1] Analyzed values.
[2] Source: Goddard, V. R., and Goodall, L. (*74*).
[a] Item 322 a. [b] Item 88 a. [c] Item 19 a. [d] Item 21 a. [e] Item 170 a.
[3] Source: Lange, W. (*112*).
[4] Source: Goodard, V. R., and Goodall, L. (*73*).
[5] Source: Del Vecchio, A., Keys, A., and Anderson, J. T. (*45*).
[6] Calculated assuming fat with composition of butterfat.
[7] Calculated from composition of fat.
[8] Unpublished data.

TABLE 77.—*Vitamin composition per 100 grams of ingredients in experimental diets*

Ingredient	Thiamine		Riboflavin		Niacin		Folic acid		Pyridoxine	
	Mg.	Data from—	Mg.	Data from—	Mg.	Data from—	Mg.	Data from—	Mg.	Data from—
Casein	0. 025	Mfr.	0. 75	Mfr.	0. 105	Mfr.	0. 015	Mfr.	0. 076	Mfr.
Lactalbumin	. 041	Unpublished	. 43	Unpublished						
Yeast	60. 0	Mfr.	7. 0	Mfr.	50. 0	Mfr.	2. 07	(*185*)	2. 05	Mfr.
Egg, dried	. 34	(*188*)	1. 06	(*188*)	. 20	(*188*)	. 012	(*185*)	. 544	(*154*, table IV).
Egg yolk, dried	. 50	(*188*)	. 66	(*188*)	. 10	(*188*)	. 026	(*185*)	1. 18	(*154*, table IV).
Egg white, dried			2. 05	(*188*)	. 70	(*188*)	. 003	(*185*)	. 058	(*154*, table IV).
Skim milk solids	. 35	(*188*)	1. 96	(*188*)	1. 10	(*188*)	. 003	(*185*)	. 040	(*184*)
Butter			. 01	(*188*)	. 10	(*188*)				
Beef	. 18	(*188*)	. 49	(*188*)	12. 3	(*188*)	. 017	(*185*)	1. 53	(*184*)
Peanut butter	. 12	(*188*)	. 13	(*188*)	16. 2	(*188*)	. 052	(*185*)	. 40	(*184*)
Kale	. 10	(*188*)	. 26	(*188*)	2. 0	(*188*)				

Ingredient	Pantothenic acid		Vitamin B_{12}		Choline		Biotin		Vitamin A	
	Mg.	Data from—	µg.	Data from—	Mg.	Data from—	µg.	Data from—	I.U.	Data from—
Casein	0. 042	Mfr.	6. 6	(*52*, p. 97).	Trace	(*56*)	2. 0	Mfr.		
Lactalbumin										
Yeast	12. 5	Mfr.			262	(*96*, p. 332).	83	(*110*, table 2).		
Egg, dried	7. 34	(*193*)	10. 0	(*116*)	1, 864	(*56*)	45	(*110*, table 2).	3, 740	(*188*)
Egg yolk, dried	7. 92	(*193*)	14. 7	(*116*)	2, 740	(*56*)	44	(*110*, table 2).	5, 540	(*188*)
Egg white, dried	1. 14	(*193*)			Trace	(*56*)	48	(*110*, table 2).		
Skim milk solids	3. 36	(*193*)	3. 76	(*116*)	138	(*56*)	35	(*95*, table 1)	40	(*188*)
Butter					Trace	(*56*)			3, 300	(*188*)
Beef	1. 76	(*193*)	7. 00	(*116*)	281	(*56*)	13	(*110*, table 2).		
Peanut butter	2. 14	(*193*)			126	(*56*)	40	(*110*, table 2).		
Kale									7, 540	(*188*)

TABLE 78.—*Ash*[1] *and mineral composition per 100 grams of ingredients in experimental diets*

Food	Ash	Calcium	Phosphorus	Iron	Copper	Sodium	Potassium	Magnesium	Manganese	Boron
	Grams	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Salt mixture	------	12, 700	7, 580	290	6. 4	3, 530	18, 800	1, 540	6. 7	------
Casein	1. 40	91	420	. 69	. 27	3. 8	[2] ND	11	. 07	0. 16
Lactalbumin	1. 16	80	170	4. 0	2. 1	160	200	16	. 02	. 13
Yeast	7. 52	110	1, 100	8. 1	3. 6	23	2, 100	200	1. 8	. 10
Egg, dried	3. 44	210	620	7. 5	. 37	400	500	42	. 18	. 17
Egg yolk, dried	3. 88	240	720	8. 5	. 18	110	130	16	. 13	. 10
Egg white, dried	7. 65	87	92	. 54	. 22	880	900	92	. 02	. 08
Skim milk solids	4. 44	1, 100	670	. 31	. 06	430	1, 650	66	. 04	. 10
Beef, dried	8. 29	110	520	15. 0	. 37	230	1, 000	88	. 07	. 12
Peanut butter	1. 50	24	300	2. 2	. 48	1. 1	810	300	1. 6	. 60

[1] All analytically determined values, except salt mixture, calculated from ingredients. Spectrochemical analyses for minerals carried out by A. W. Specht and J. W. Resnicky, Soil and Water Conservation Research Division, Agricultural Research Service, Beltsville, Md.

[2] Not detectable; limit of sensitivity for potassium 0.03 per 100 grams.

TABLE 79.—*Weekly weight gain and food intake of rats fed SP 8 HVO, SPE, SPM, SPB, and SPPB diets for first 12 weeks*

Strain, diet, and series	Rats	Weaning weight [1]	Week 1			Week 2			Week 3			Week 4			Week 5			Week 6		
			Gain	Intake	Calories	Gain	Intake	Calories	Gain	Intake	Calories	Gain	Intake	Calories	Gain	Intake	Calories	Gain	Intake	Calories
BHE rats																				
SP 8 HVO:	*Number*	*Grams*	*Grams*	*Grams*		*Grams*	*Grams*		*Grams*	*Grams*		*Grams*	*Grams*		*Grams*	*Grams*		*Grams*	*Grams*	
1	14	46	34	61	288	44	80	376	43	96	452	41	105	495	41	119	560	34	118	555
A	10	44	34	62	293	37	80	378	38	92	433	36	103	483	37	105	493	32	111	523
B	10	46	37	58	274	43	77	361	40	94	440	39	102	478	40	110	517	30	116	547
K	10	44	38	60	284	38	73	344	39	87	408	40	100	471	39	109	514	32	110	519
Average	44	45	35	61	285	41	78	365	40	92	435	39	103	483	39	112	524	32	114	538
SPE:																				
1	15	47	37	54	281	45	70	364	44	83	432	47	94	488	48	116	551	37	106	551
I	8	45	34	54	281	43	75	390	44	91	473	46	98	510	48	104	541	43	109	567
J	10	48	40	59	307	44	80	416	42	85	442	42	87	452	41	98	510	38	99	515
F	5	43	39	56	291	45	75	390	43	88	458	41	90	468	43	101	525	38	105	546
Average	38	46	37	56	289	44	74	387	44	86	447	45	92	481	46	103	535	39	105	544
Wistar rats																				
SP 8 HVO	10	48	27	56	265	39	77	360	41	89	420	38	93	437	34	95	445	34	103	484
SPE	10	48	30	51	263	44	70	364	48	84	434	43	87	454	41	90	470	36	94	488
BHE littermates																				
SP 8 HVO	24	47	33	59	278	45	80	375	44	97	458	42	108	510	42	122	572	36	123	577
SPE	24	47	37	55	287	46	71	368	45	85	443	47	96	498	47	105	547	37	107	554
SPM	24	47	36	53	263	42	73	361	43	90	443	44	100	492	44	112	550	40	118	583
SPB	24	47	38	53	275	46	71	369	45	86	445	44	93	484	44	104	541	37	107	557
SPPB	24	47	38	54	267	46	74	367	45	91	452	47	98	489	49	110	546	41	114	570

Strain, diet, and series	Week 7			Week 8			Week 9			Week 10			Week 11			Week 12			Total for 12 weeks		
	Gain	Intake	Calories	Gain	Intake	Calories	Gain	Intake	Calories	Gain	Intake	Calories	Gain	Intake	Calories	Gain	Intake	Calories	Gain	Intake	Calories
BHE rats																					
SP 8 HVO:	*Grams*	*Grams*		*Grams*	*Grams*		*Grams*	*Grams*		*Grams*	*Grams*		*Grams*	*Grams*		*Grams*	*Grams*		*Grams*	*Grams*	
1	29	128	601	28	128	601	22	125	587	17	121	568	16	120	565	13	123	576	362	1,324	6,220
A	32	116	544	29	117	552	25	121	567	22	115	541	15	116	543	16	119	559	353	1,257	5,910
B	28	118	552	27	117	548	22	115	539	15	112	526	12	108	510	14	114	534	345	1,240	5,830
K	25	107	502	25	114	534	22	109	513	19	114	538	18	118	553	16	122	573	350	1,224	5,750
Average	28	118	555	27	120	563	22	118	555	18	116	545	15	116	545	15	120	562	353	1,267	5,950
SPE:																					
1	32	111	577	32	115	598	24	114	593	23	115	598	21	112	582	17	113	588	406	1,193	6,200
I	37	110	571	30	114	593	31	117	608	27	111	577	22	111	577	18	108	562	423	1,202	6,250
J	29	109	567	26	105	546	24	107	556	20	105	546	20	106	551	20	108	562	386	1,148	5,970
F	32	106	551	23	107	556	20	102	530	17	111	577	15	106	551	15	98	510	371	1,145	5,950
Average	32	110	570	29	111	578	25	111	578	22	111	578	20	109	569	18	109	565	400	1,177	6,120
Wistar rats																					
SP 8 HVO	28	100	471	23	101	477	19	97	454	16	98	458	14	96	451	12	95	446	324	1,100	5,170
SPE	29	91	474	27	92	479	20	92	476	16	92	479	12	87	452	17	91	471	364	1,020	5,300
BHE littermates																					
SP 8 HVO	29	127	599	28	130	609	22	126	590	20	126	594	17	124	584	15	125	586	373	1,347	6,330
SPE	33	111	579	30	114	592	24	114	592	22	113	589	20	112	584	16	111	579	404	1,192	6,200
SPM	35	122	599	31	121	599	26	122	602	25	124	609	21	120	592	18	121	599	405	1,277	6,300
SPB	34	112	582	29	113	589	26	112	584	23	113	587	18	109	566	16	109	566	400	1,182	6,140
SPPB	33	114	570	29	115	575	25	116	577	22	114	568	20	114	567	18	115	575	412	1,229	6,120

[1] Average weaning age—22 days (21 to 26).

TABLE 80.—*Weight, weight gain, and food intake of rats for 100-day intervals on SP 8 HVO, SPE, SPM, SPB, and SPPB diets*

Strain, diet, and series	Rats	Weight at 100 days	100–200 days Gain	100–200 days Intake	100–200 days Calories	Weight at 200 days	200–300 days Gain	200–300 days Intake	200–300 days Calories	Weight at 300 days	300–400 days Gain	300–400 days Intake	300–400 days Calories	Weight at 400 days
BHE rats														
SP 8 HVO:	*Number*	*Grams*	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*		*Grams*
1	14	399	95	1,736	8,160	494	[1] 52 (13)	1,796	8,440	551	33 (10)	1,764	8,290	584
A	10	387	129	1,622	7,620	516	40	1,674	7,870	556	48	1,707	8,020	605
B	10	378	109	1,718	8,080	487	66	1,733	8,140	553	54	1,704	8,010	607
K	10	379	142	1,765	8,300	521	60	1,684	7,920	581	−9 (9)	1,655	7,780	569
Average	44	387	117	1,713	8,050	504	54 (43)	1,727	8,120	560	33 (39)	1,715	8,060	591
SPE:														
1	15	439	112	1,616	8,400	552	53	1,654	8,600	605	22 (5)	1,490	7,750	572
I	8	468	100	1,514	7,870	568	26 (7)	1,523	7,920	605	60 (5)	1,817	9,450	683
J	10	415	119	1,492	7,760	534	75	1,592	8,280	609	18 (3)	1,633	8,490	638
F	5	404	125	1,336	6,950	529	92	1,418	7,370	621	3 (4)	1,355	7,050	627
Average	38	434	113	1,525	7,930	547	59 (37)	1,581	8,220	608	30 (17)	1,580	8,220	629
Wistar rats														
Littermates fed—														
SP 8 HVO	10	363	92	1,358	6,380	455	17	1,255	5,900	472	21	1,355	6,270	493
SPE	10	398	117	1,319	6,860	515	30 (8)	1,286	6,690	550	37 (7)	1,336	6,950	587
BHE rats														
Littermates fed—														
SP 8 HVO	14	399	95	1,736	8,160	494	52 (13)	1,796	8,440	551	33 (10)	1,764	8,290	584
SPE	14	438	109	1,607	8,360	547	51	1,647	8,560	598	20 (4)	1,475	7,670	574
SPM	14	429	134 (13)	1,693	8,350	556	66	1,701	8,390	622	46 (11)	1,740	8,580	662
SPB	14	430	108	1,574	8,180	538	71	1,608	8,360	609	34 (10)	1,614	8,390	632
SPPB	14	442	128 (13)	1,636	8,150	568	63 (12)	1,660	8,270	618	44 (9)	1,646	8,200	653

Strain, diet, and series	400–500 days Gain	400–500 days Intake	400–500 days Calories	Weight at 500 days	500–600 days Gain	500–600 days Intake	500–600 days Calories	Weight at 600 days	600–700 days Gain	600–700 days Intake	600–700 days Calories	Weight at 700 days	700–800 days Gain	700–800 days Intake	700–800 days Calories	Weight at 800 days
BHE rats																
SP 8 HVO:	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*		*Grams*
1	12 (7)	1,824	8,570	566	30 (3)	1,728	8,120	542	14 (2)	1,742	8,190	574	6 (1)	1,740	8,180	593
A	48 (8)	1,759	8,270	666	20 (6)	1,783	8,380	674	30 (2)	1,900	8,930	628	30 (1)	1,795	8,440	678
B	18 (7)	1,763	8,290	620	39 (6)	1,899	8,920	669	—	—	—	—	—	—	—	—
K	10 (6)	1,650	7,760	568	40 (4)	1,826	8,580	572	33 (3)	1,728	8,130	588	—	—	—	—
Average	23 (28)	1,753	8,240	608	32 (19)	1,835	8,620	628	26 (7)	1,781	8,370	595	18 (2)	1,768	8,310	636
SPE:																
1	4 (2)	1,450	7,540	515	36 (1)	1,400	7,280	550	—	—	—	—	—	—	—	—
I	19 (3)	1,729	8,990	677	—	—	—	—	—	—	—	—	—	—	—	—
J	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F	1 (3)	1,314	6,830	627	—	—	—	—	—	—	—	—	—	—	—	—
Average	8 (8)	1,504	7,820	618	36 (1)	1,400	7,280	550	—	—	—	—	—	—	—	—
Wistar rats																
Littermates fed—																
SP 8 HVO	23 (8)	1,349	6,340	518	37 (7)	1,441	6,770	548	−5 (5)	1,451	6,820	564	42 (2)	1,530	7,190	599
SPE	78 (7)	1,102	7,290	665	64 (6)	1,438	7,480	688	18 (6)	1,463	7,610	706	21 (3)	1,611	8,380	771
BHE rats																
Littermates fed—																
SP 8 HVO	12 (7)	1,824	8,570	566	30 (3)	1,728	8,120	542	14 (2)	1,742	8,190	574	6 (1)	1,740	8,180	593
SPE	4 (2)	1,450	7,540	515	36 (1)	1,400	7,280	550	—	—	—	—	—	—	—	—
SPM	11 (7)	1,832	9,030	659	70 (6)	1,901	9,370	732	43 (6)	1,885	9,290	775	—	—	—	—
SPB	25 (5)	1,773	9,220	665	22 (4)	1,797	9,340	667	61 (2)	1,813	9,430	726	—	—	—	—
SPPB	27 (6)	1,739	8,660	664	59 (5)	1,833	9,130	728	17 (2)	1,864	9,280	810	—	—	—	—

[1] Rats consistently losing weight not included. Numbers in parentheses indicate rats still maintaining or gaining weight.

TABLE 81.—*Body weight, food intake, survival, and organ weights of individual BHE nonfasted rats fed stock and SPE diets throughout life or reversed at 250 days*

Condition and diet	Litter No.	Weight at—			Weight gain at—		Food intake at—		Gross energy at—		Age at death	Weight at death	Maximum weight	Weight of selected organs			
		200 days	250 days	300 days	200–250 days	250–300 days	200–250 days	250–300 days	200–250 days	250–300 days				Liver	Kidney	Adrenal	Thyroid
Sacrificed at 250 days:		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Calories*	*Calories*	*Days*	*Gra*	*Grams*	*Grams*	*Grams*	*Mg.*	*Mg.*
Stock	1	487	516	----	29	----	1,009	----	4,030	----	248	516	----	18.00	1.72	23.9	11.9
	2	446	456	----	10	----	963	----	3,840	----	251	456	----	15.57	1.70	17.5	12.5
	4	529	536	----	7	----	1,013	----	4,040	----	250	536	----	17.29	2.80	27.4	12.7
Average	----	487	503	----	15	----	995	----	3,970	----	250	503	----	16.95	2.07	22.9	12.4
SPE	1	484	543	----	59	----	741	----	3,850	----	248	543	----	22.86	1.77	20.5	20.8
	2	556	527	----	−29	----	789	----	4,100	----	249	527	----	28.28	4.19	26.2	15.3
	4	615	652	----	37	----	829	----	4,310	----	249	660	----	28.15	2.26	22.4	14.5
Average	----	552	574	----	22	----	786	----	4,090	----	249	577	----	26.43	2.74	23.0	16.9
Continued on—																	
Stock	1	448	504	511	56	7	969	933	3,870	3,720	623	356	528	14.58	2.76	----	----
	3	479	491	529	12	38	959	1,004	3,830	4,010	420	367	537	20.47	5.04	26.0	14.6
	5	530	587	633	57	46	1,213	1,200	4,840	4,790	727	453	697	20.48	4.96	25.3	28.4
Average	----	486	527	558	42	30	1,047	1,046	4,180	4,170	590	392	587	18.51	4.25	25.6	21.5
SPE	1	470	503	522	13	19	658	626	3,420	3,260	392	365	530	13.75	4.17	38.2	17.1
	3	542	557	578	15	21	745	721	3,870	3,750	399	477	578	19.71	5.11	22.8	17.4
	5	529	614	697	85	83	774	826	4,020	4,400	629	779	825	33.28	3.22	----	----
Average	----	514	558	599	44	41	726	724	3,770	3,800	473	540	644	22.25	4.17	30.5	17.2
Reversed at 250 days:																	
Stock changed to SPE	2	450	469	560	19	91	1,066	877	4,250	4,560	805	596	727	19.52	2.68	37.3	21.6
	3	548	566	646	18	80	1,173	898	4,680	4,670	532	483	648	20.85	4.70	40.2	13.0
	4	481	489	591	8	102	1,029	887	4,110	4,610	701	469	608	17.58	4.18	32.8	22.8
	5	533	558	645	25	87	1,122	898	4,480	4,670	705	791	791	31.08	4.22	23.2	21.3
Average	----	503	520	610	18	90	1,098	890	4,380	4,630	686	585	694	22.26	3.94	33.4	19.7
SPE changed to stock	2	535	530	456	−5	−74	761	904	3,960	3,610	384	384	535	24.24	4.38	24.7	21.3
	3	554	598	613	44	15	766	1,024	3,980	4,090	767	632	688	----	3.26	----	----
	4	587	628	612	41	−16	815	1,044	4,240	4,170	707	466	643	18.94	3.89	40.2	23.6
	5	606	709	684	103	−25	906	944	4,710	3,770	640	689	709	21.98	2.39	26.1	22.3
Average	----	570	616	591	46	−25	812	979	4,220	3,910	624	543	644	21.72	3.48	30.3	22.4

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